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PTO/SB/05 (12/97)
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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b)

Attorney Docket No. B98-031-5

First Named Inventor or Application Identifier Goodman et al.

Title Modulating Robo: Ligand Interactions

Express Mail Label No. EL071088080US

EL071088080US

ADDRESS TO: **Assistant Commissioner for Patents**
Box Patent Application
Washington, D. C. 20231

APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents.

1. X *Fee Transmittal Form
(Submit an original, and a duplicate for fee processing)

2. X Specification (Total Pages 33)
(preferred arrangement set forth below)
- Descriptive Title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claims
- Abstract of the Disclosure

3. _ Drawings(s) (35 USC 113) (Total Sheets ____)

4. X Oath or Declaration (Total Pages 2)
   a. ___ Newly Executed (Original or Copy)
   b. X Copy from a Prior Application (37 CFR 1.63(d))
   (for Continuation/Divisional with Box 17 completed)
   i. ___ DELETIONS OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).

5. X Incorporation By Reference
The entire disclosure of the prior application is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. _ Microfiche Computer Program (Appendix)
7. X Nucleotide and/or Amino Acid Sequence Submission

(if applicable, all necessary)

a.	____	Computer Readable Copy
b.	_x_	Paper Copy (identical to computer copy)
c.	____	Statement verifying identity of above copies
d.	_x_	Request to use CRF from another application

ACCOMPANYING APPLICATION PARTS

8. X Assignment Papers (cover sheet & documents(s))
   _ a. Assignment to The Regents of the Unversity of California, of record in prior application
9. X 37 CFR 3.73(b) Statement (where there is an assignee)
   X Power of Attorney (copy from prior application)
10. _ English Translation Document (if applicable)
11. X a. Information Disclosure Statement (IDS)/PTO-1449
    _ b. Copies of IDS Citations
12. X Preliminary Amendment
13. X Return Receipt Postcard (MPEP 503) (Should be specifically itemized)
14. X a. *Small Entity Statement(s) (copy from prior application)
    X b. Statement filed in prior application, Status still proper and desired
15. _ Certified Copy of Priority Document(s) (if foreign priority is claimed)
16. _ Other: ____________________

*NOTE FOR ITEMS 1 & 14: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 CFR 1.27) , EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 CFR 1.28)

17. **Priority**

This application claims priority to prior application No: 09/191,647

Prior application information: Examiner Terry McKelvey Group Art Unit 1636

18. **Correspondence Address**

23379

PATENT TRADEMARK OFFICE

_____ Customer Number or Bar Code Label ____________ (Insert Customer No. or Attach Bar Code Label here)

or

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Signature: [signature] Date: March 31, 2000

Docket No. B98-031-3

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(d)) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: The Regents of the University of California
ADDRESS: 1111 Franklin Street, 5th Floor, Oakland, CA 94607-5200

TYPE OF ORGANIZATION
University or other Institution of Higher Education

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) or (b) of Title 35, United States Code, with regard to the invention entitled *Modulating Robo: Ligand Interactions* by inventors Corey S. Goodman, Thomas Kidd, Katja Brose and Marc Tessier-Lavigne described in the application filed on November 13, 1998 having USSN **09/191,647**.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization identified above with regard to the invention entitled *Modulating Robo: Ligand Interactions*, and having the named inventor(s): Goodman et al. described in the Application filed on November 13, 1998 having USSN **09/191,647**. If the rights held by the above identified nonprofit organization are not exclusive, each individual, concern or organization having rights in the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

Name: ________________________________
Address: ________________________________

[ ] Individual [ ] Small Business Concern [ ] Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name/Title: William A. Hoskins, Director, Office of Technology Licensing
Address: Office of Technology Licensing, 2150 Shattuck Ave., Berkeley, CA 94704

SIGNATURE ________________ DATE Feb 11, 1999

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Goodman et al.

Serial No. Not yet assigned

Filed: Herewith

For: *Modulating Robo: Ligand Interactions*

Group Art Unit: 1636

Examiner: McKelvey, T.

Attorney Docket No. B98-031-5

Date: March 31, 2000

This is a divisional application of US Serial No. 09/191,647, filed November 13, 1998.

PRELIMINARY AMENDMENT

The Assistant Commissioner for Patents
Washington, DC 20231

Dear Commissioner:

Please enter the following preliminary amendments in this divisional application:

IN THE SPECIFICATION

At page 1, line 3, please delete "Inventors: Corey S. Goodman, Thomas Kidd, Katja Brose and Marc Tessier-Lavigne".

At page 1, lines 9-10, please change "is a continuing ... Nov 14 1997" to --claims the benefit of U.S. Application No. 09/191,647, filed November 13, 1998, which claims the benefit of U.S. Provisional Application No. 60/081,057 filed Apr 07, 1998 and U.S. Provisional Application No. 60/065,544, filed Nov 14, 1997--.

At page 6, line 17, immediately following "Tables 3 and 4.", please insert the attached Tables 1 and 2, and please change "white backgrounded sequences in Tables 3 and 4" to --unboxed sequences in Tables 1 and 2--. Also, please insert page numbers on the pages of the attached Tables 1 and 2 corresponding to their position in the specification and please renumber the subsequent pages of the specification accordingly.

At page 6, line 18, please change "Table 1" to --Table 3--.

At page 6, line 10, please change "fragemtns" to --fragments--.

At page 6, line 20, please change "Table 1" to --Table 3--.

At page 7, line 24, please change "Table 2" to --Table 4--.

At page 8, line 1, please change "Table 2" to --Table 4--.

At page 11, lines 21-22, please change "Table 5 (A and B)" to --Table 5--.

At page 11, immediately before line 23, please insert the following text:

--Table 5. Hybridization Probes for Regions of Human Slit-1.

Hybridization probe for first leucine rich repeat region	SEQ ID NO:01, nucleotides 82-828
Hybridization probe for second leucine rich repeat region	SEQ ID NO:01, nucleotides 829-1503
Hybridization probe for third leucine rich repeat region	SEQ ID NO:01, nucleotides 1504-2166
Hybridization probe for fourth leucine rich repeat region	SEQ ID NO:01, nucleotides 2167-2751
Hybridization probe for EGF repeats one to five	SEQ ID NO:01, nucleotides 2752-3327
Hybridization probe for the sixth EGF repeat and preceding spacer region	SEQ ID NO:01, nucleotides 3328-3461
Hybridization probe for the 99aa spacer/G-loop region	SEQ ID NO:01, nucleotides 3462-3987
Hybridization probe for EGF repeats seven to nine	SEQ ID NO:01, nucleotides 3988-4341
Hybridization probe for the cysteine knot region	SEQ ID NO:01, nucleotides 4342-4575

Table 6. PCR Primers for regions of Human Slit.

PCR Primers for first leucine rich repeat region	Forward: SEQ ID NO:01, nucleotides 82-111 Reverse: reverse complement of SEQ ID NO:01, nucleotides 799-828
PCR Primers for second leucine rich repeat region	Forward: SEQ ID NO:01, nucleotides 829-858 Reverse: reverse complement of SEQ ID NO:01, nucleotides 1474-1503

PCR Primers for third leucine rich repeat region	Forward: SEQ ID NO:01, nucleotides 1504-1533 Reverse: reverse complement of SEQ ID NO:01, nucleotides 2137-2166
PCR Primers for fourth leucine rich repeat region	Forward: SEQ ID NO:01, nucleotides 2167-2196 Reverse: reverse complement of SEQ ID NO:01, nucleotides 2722-2751
PCR Primers for EGF repeats one to five	Forward: SEQ ID NO:01, nucleotides 2752-2781 Reverse: reverse complement of SEQ ID NO:01, nucleotides 3298-3327
PCR Primers for the sixth EGF repeat and preceding spacer region	Forward: SEQ ID NO:01, nucleotides 3328-3357 Reverse: reverse complement of SEQ ID NO:01, nucleotides 3432-3461
PCR Primers for the 99aa spacer/G-loop region	Forward: SEQ I:01, nucleotides 3462-3491 Reverse: reverse complement of SEQ ID NO:01, nucleotides 3958-3987
PCR Primers for EGF repeats seven to nine	Forward: SEQ ID NO:01, nucleotides 3988-4017 Reverse: reverse complement of SEQ ID NO:01, nucleotides 4312-4341
PCR Primers for the cysteine knot region	Forward: SEQ ID NO:01, nucleotides 4342-4371 Reverse: reverse complement of SEQ ID NO:01, nucleotides 4546-4575

Leucine rich repeats (LRRs) are predicted by comparison with known proteins and by the presence of a leucine rich core sequence. In slit proteins, the LRRs are flanked by conserved sequences referred to as the amino- and carboxy- flanking regions. These flanking regions are found in other known proteins, but only in a few instances are both the amino- and carboxy- flank regions present in a single protein. The so called "99aa spacer" is actually ~200 amino acids in the Drosophila protein and 174 amino acids in Human Slit-1. This region shows homology to the G-loops of laminin A chains.

Cysteine knots are dimerisation domains defined by the presence of six cysteine residues between which disulphide bridges form. The only absolutely conserved residues are the six cysteines, and spacing between them is highly variable, apart from between cysteines 2 and 3, and 5 and 6. The glycine between cysteines 2 and 3 is only present in a subset of cysteine knots.

Drosophila slit and Human slit-1 both have an extra cysteine after cysteines 5 and 6: this may serve as an intermolecular bond. Human Slit-1 gene displays the overall structure of the Drosophila gene, and amino acid conservation is found along the entire length of the protein (48% homology at the amino acid sequence excluding the signal sequence; see below). The Human gene has an extra LRR between LRR2 and LRR3 of the first set of LRRs; in the third set, the Human gene has an extra LRR between LRR3 and LRR4. The Human gene has two extra EGF repeats, on either side of the seventh EGF repeat in Drosophila slit.

Isolation of Human slit-1

Searching of the EST database revealed an EST, ab16g10.r1, with homology to the 99aa spacer region of Drosophila slit. This EST was used to probe a Human fetal brain library (Stratagene), and clones for Human slit-1 were isolated.

Features of Human Slit Predicted Protein

Signal sequence	SEQ ID NO:02, residues 7-24
First amino-flanking sequence	SEQ ID NO:02, residues 28-59
First set of Leucine Rich Repeats	SEQ ID NO:02, residues 60-179 (6 repeats)
First carboxy-flanking sequence	SEQ ID NO:02, residues 180-276
Second amino-flanking sequence	SEQ ID NO:02, residues 277-308
Second set of Leucine Rich Repeats	SEQ ID NO:02, residues 309-434 (5 repeats)
Second carboxy-flanking sequence	SEQ ID NO:02, residues 435-501
Third amino-flanking sequence	SEQ ID NO:02, residues 502-533
Third set of Leucine Rich Repeats	SEQ ID NO:02, residues 534-560 (5 repeats)
Third carboxy-flanking sequence	SEQ ID NO:02, residues 661-722
Fourth amino-flanking sequence	SEQ ID NO:02, residues 723-754
Fourth set of Leucine Rich Repeats	SEQ ID NO:02, residues 755-855 (4 repeats)
Fourth carboxy-flanking sequence	SEQ ID NO:02, residues 856-917
First EGF repeat	SEQ ID NO:02, residues 918-952
Second EGF repeat	SEQ ID NO:02, residues 953-993
Third EGF repeat	SEQ ID NO:02, residues 994-1031

Fourth EGF repeat	SEQ ID NO:02, residues 1032-1071
Fifth EGF repeat	SEQ ID NO:02, residues 1072-1109
Spacer	SEQ ID NO:02, residues 1110-1116
Sixth EGF repeat	SEQ ID NO:02, residues 1117-1153
"99aa spacer"	SEQ ID NO:02, residues 1155-1329
Seventh EGF repeat	SEQ ID NO:02, residues 1330-1366
Eighth EGF repeat	SEQ ID NO:02, residues 1367-1404
Nineth EGF repeat	SEQ ID NO:02, residues 1405-1447
Cysteine knot motif	SEQ ID NO:02, residues 1448-1525

Amino acid identity between Drosophila and Human Slit-1

First amino-flanking sequence	53%
First set of Leucine Rich Repeats	52% (54%, 67%, NA, 38%, 54%, 50%)
First carboxy-flanking sequence	42%
Second amino-flanking sequence	50%
Second set of Leucine Rich Repeats	60% (54%, 58%, 67%, 71%, 50%)
Second carboxy-flanking sequence	62%
Third amino-flanking sequence	56%
Third set of Leucine Rich Repeats	49% (46%, 46%, 42%, NA, 58%)
Third carboxy-flanking sequence	36%
Fourth amino-flanking sequence	53%
Fourth set of Leucine Rich Repeats	48% (25%, 58%, 46%, 63%)
Fourth carboxy-flanking sequence	63%
First EGF repeat	34%
Second EGF repeat	46%
Third EGF repeat	46%
Fourth EGF repeat	35%
Fifth EGF repeat	47%

Spacer	22%
Sixth EGF repeat	40%
"99aa spacer"	38%
Seventh EGF repeat	11% /NA
Eighth EGF repeat	44%
Nineth EGF repeat	29% /NA
Cysteine knot motif	34%

NA: not applicable due to absence of homologous repeat.
Figures for individual LLRs are shown in brackets.--

Immediately prior to the claims, please insert the enclosed 23 page section entitled "SEQUENCE LISTING".

Please delete all pages after page 17.

IN THE CLAIMS

Please cancel all pending claims (1-7) and add new claims 8-27 as follows:

8. (New) A mixture comprising an isolated Slit polypeptide and a Robo polypeptide, said Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:2-14, or a subsequence thereof having at least 16 consecutive amino acid residues thereof.

9. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:2-14, or a subsequence thereof having at least 64 consecutive amino acid residues thereof.

10. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:2-14.

11. (New) A mixture according to claim 8, the Slit polypeptide comprising SEQ ID NO:2, or a subsequence thereof having at least 16 consecutive amino acid residues thereof.

12. (New) A mixture according to claim 8, the Slit polypeptide comprising SEQ ID NO:2, or a subsequence thereof having at least 64 consecutive amino acid residues thereof.

13. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:3-6, or a subsequence thereof having at least 16 consecutive amino acid residues thereof.

14. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:3-6, or a subsequence thereof having at least 64 consecutive amino acid residues thereof.

15. (New) A mixture according to claim 8, the Slit polypeptide comprising SEQ ID NO:7, or a subsequence thereof having at least 16 consecutive amino acid residues thereof.

16. (New) A mixture according to claim 8, the Slit polypeptide comprising SEQ ID NO:7, or a subsequence thereof having at least 64 consecutive amino acid residues thereof.

17. (New) A mixture according to claim 8, the Slit polypeptide at comprising least one sequence selected from the group consisting of SEQ ID NOS:8-9, or a subsequence thereof having at least 16 consecutive amino acid residues thereof.

18. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:8-9, or a subsequence thereof having at least 64 consecutive amino acid residues thereof.

19. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:10-11, or a subsequence thereof having at least 16 consecutive amino acid residues thereof.

20. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:10-11, or a subsequence thereof having at least 64 consecutive amino acid residues thereof.

21. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:12-14, or a subsequence thereof having at least 16 consecutive amino acid residues thereof.

22. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NOS:12-14, or a subsequence thereof having at least 64 consecutive amino acid residues thereof.

23. (New) A mixture according to claim 8, the Slit polypeptide comprising at least one sequence selected from the group consisting of SEQ ID NO:2, amino acid residues 1-10; SEQ ID NO:2, amino acid residues 29-41; SEQ ID NO:2, amino acid residues 75-87; SEQ ID NO:2, amino acid residues 92-109; SEQ ID NO:2, amino acid residues 132-141; SEQ ID NO:2, amino acid residues 192-205; SEQ ID NO:2, amino acid residues 258-269; SEQ ID NO:2, amino acid residues 295-311; SEQ ID NO:2, amino acid residues 316-330; SEQ ID NO:2, amino acid residues 373-382; SEQ ID NO:2, amino acid residues 403-422; SEQ ID NO:2, amino acid residues 474-485; SEQ ID NO:2, amino acid residues 561-576; SEQ ID NO:2, amino acid residues 683-697; SEQ ID NO:2, amino acid residues 768-777; SEQ ID NO:2, amino acid residues 798-813; SEQ ID NO:2, amino acid residues 882-894; SEQ ID NO:2, amino acid residues 934-946; SEQ ID NO:2, amino acid residues 1054-1067; SEQ ID NO:2, amino acid residues 1181-1192; SEQ ID NO:2, amino acid residues 1273-1299; SEQ ID NO:2, amino acid residues 1383-1397; SEQ ID NO:2, amino acid residues 1468-1477; and SEQ ID NO:2, amino acid residues 1508-1517.

24. (New) A mixture according to claim 8, comprising a cell comprising the Robo polypeptide.

24. (New) A mixture according to claim 10, comprising a cell comprising the Robo polypeptide.

25. (New) A mixture according to claim 8, comprising a candidate agent for modulating an interaction of the Robo and Slit polypeptides.

26. (New) A method of identifying agents which modulate the interaction of a Robo polypeptide and a Slit polypeptide, said method comprising the steps of:

combining the mixture of claim 8 and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, and

determining a second interaction of the Robo and Slit polypeptides in the presence of the agent,

wherein a difference between the first and second interactions indicates that the agent modulates the interaction of the Robo and Slit polypeptides.

27. (New) A method of identifying agents which modulate the interaction of a Robo polypeptide and a Slit polypeptide, said method comprising the steps of:

combining the mixture of claim 8 and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, and

determining a second interaction of the Robo and Slit polypeptides in the presence of the agent,

wherein a difference between the first and second interactions indicates that the agent modulates the interaction of the Robo and Slit polypeptides.

REMARKS

The foregoing amendments to the specification are identical to those made in the parent application Serial No.: 09/191,647 except update the "Cross Reference to Related Application" section of the instant application.

As explained in 09/191,647, these amendments to the specification are intended to address Sequence Listing formalities and to incorporate the sections appended to the application as filed:

(1) by relocating the bodies and headings of Tables 3 and 4 (appended to the specification as filed) to page 6, renumbering them Tables 1 and 2 respectively and reformatting the shaded areas as open boxes.

(2) by renumbering Tables 1 and 2 as filed, as Tables 3 and 4 respectively.

(3) by relocating Tables 5 (A-B) and 6 (appended to the specification as filed) and the text accompanying these tables to page 11, and renumbering Table 5 (A-B) as Table 5.

(4) by relocating the sections entitled "Features of Human Slit Predicted Protein" and "Amino acid identity between Drosophila and Human Slit-1" (appended to the specification as filed) to follow Table 6 and replacing the phrase, "presence of the core sequence ... amino acid" with –presence of a leucine rich core sequence–, deleting the four sentences "The amino flank region ... Cxxxxxx." and deleting ": C[x]C[1-3x]GxC[x]C[x]CxC" in the text of the section entitled "Features of Human Slit Predicted Protein".

(5) by relocating the data of "SEQ ID NO:1 & 2" (appended to the specification as filed) to a section entitled "SEQUENCE LISTING" immediately prior to the claims. The sequences disclosed in this sequence listing are identical to those disclosed in the deleted "SEQ ID NO:1 &

2" and Tables 3 and 4, as originally filed.

In accordance with 37 CFR 1.821(e), please use the computer readable form of the Sequence Listing submitted on April 8, 1999 in Application No. 09/191,647, filed November 13, 1998 as the computer readable form of the Sequence Listing for the instant Application. It is understood that the Patent and Trademark Office will make the necessary change in Application number and filing date for the computer readable form that will be used for the instant Application. The sequence information on the written Sequence Listing enclosed herewith is identical to that recorded in computer readable form filed in the above referenced Application No. 09/191,647 and includes no new matter.

The foregoing amendments introduce no new matter.

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP

Richard Aron Osman, Ph.D., Reg. No. 36,627
Tel: (650) 343-4341; Fax: (650) 343-4342

Modulating Robo:Ligand Interactions

Inventors: Corey S. Goodman, Thomas Kidd, Katja Brose and Marc Tessier-Lavigne

The research carried out in the subject application was supported in part by NIH grant NS18366. The government may have rights in any patent issuing on this application.

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuing application under 35USC120 of USSN 60/081,057 filed Apr 07, 1998 and of USSN 60/065,544, filed Nov 14, 1997.

INTRODUCTION

Field of the Invention

The field of this invention is methods for modulating nerve cell function.

Background

In the developing CNS, most growth cones confront the midline at one or multiple times during their journey and make the decision of whether to cross or not to cross. This decision is not a static one but rather changes according to the growth cone's history. For example, in the Drosophila ventral nerve cord, about 10% of the interneurons project their axons only on their own side, in some cases extending near the midline without crossing it. The other 90% of the interneurons first project their axons across the midline and then turn to project longitudinally on the other side, often extending near the midline. These growth cones, having crossed the midline once, never cross it again, in spite of their close proximity to the midline and the many commissural axons crossing it. This decision to cross or not to cross is not unique to Drosophila but is common to a variety of midline structures in all bilaterally symmetric nervous systems.

What midline signals and growth cone receptors control whether growth cones do or do not cross the midline? After crossing once, what mechanism prevents these growth cones from crossing again? A related issue concerns the nature of the midline as an intermediate target. If so many growth cones find the midline such an attractive structure, why do they cross over it rather than linger? Why do they leave the midline?

One approach to find the genes encoding the components of such a system is to screen for mutations in which either too many or too few axons cross the midline. Such a large-scale mutant screen was previously conducted in Drosophila, and led to the identification of two key genes: *commissureless* (*comm*) and *roundabout* (*robo*) (Seeger et al., 1993; reviewed by Tear et al., 1993). In *comm* mutant embryos, commissural growth cones initially orient toward the midline but then fail to cross it and instead recoil and extend on their own side. *robo* mutant embryos, on the other hand, display the opposite phenotype in that too many axons cross the midline; many growth cones that normally extend only on their own side instead now project across the midline and axons that normally cross the midline only once instead appear to cross and recross multiple times (Seeger et al, 1993; present disclosure). Double mutants of *comm* and *robo* display a *robo*-like phenotype.

How do *comm* and *robo* function to control midline crossing? Neither the initial paper on these genes (Seeger et al., 1993) nor the cloning of *comm* (Tear et al., 1996) resolved this question. *comm* encodes a novel surface protein expressed on midline cells. In fact, the *comm* paper (Tear et al., 1996) ended with the hope that future work would "... help shed some light on the enigmatic function of Comm."

USSN 08/971,172 (*Robo, A Novel Family of Polypeptides and Nucleic Acids*, by inventors: Corey S. Goodman, Thomas Kidd, Kevin J. Mitchell and Guy Tear) discloses the cloning and characterization of *robo* in various species including Drosophila; Robo polypeptides and polypeptide-encoding nucleic acids are also disclosed and their genbank accession numbers referenced in Kidd et al. (1998) Cell 92, 205-215. *robo* encodes a new class of guidance receptor with 5 immunoglobulin (Ig) domains, 3 fibronectin type III domains, a transmembrane domain, and a long cytoplasmic domain. Robo defines a new subfamily of Ig superfamily proteins that is highly conserved from fruit flies to mammals. The Robo ectodomains, and in particular the first two Ig domains, are highly conserved from fruit fly to human, while the cytoplasmic domains are more divergent. Nevertheless, the cytoplasmic domains contain three highly conserved short proline-rich motifs which may represent binding sites for SH3 or other binding domains in linker or signaling molecules.

For those axons that never cross the midline, Robo is expressed on their growth cones from the outset; for the majority of axons that do cross the midline, Robo is expressed at high levels on their growth cones only after they cross the midline. Transgenic rescue experiments

in Drosophila reveal that Robo can function in a cell autonomous fashion, consistent with it functioning as a receptor. Thus, in Drosophila, Robo appears to function as the gatekeeper controlling midline crossing; growth cones expressing high levels of Robo are prevented from crossing the midline. Robo proteins in mammals function in a similar manner in controlling axon guidance.

USSN 60/065,54 (*Methods for Modulating Nerve Cell Function*, by inventors: Corey S. Goodman, Thomas Kidd, Guy Tear, Claire Russell and Kevin Mitchell) discloses ectopic and overexpression studies revealing that Comm down-regulates Robo expression, demonstrating that Comm functions to suppress the Robo-mediated midline repulsion. These results show that the levels of Comm at the midline and Robo on growth cones are tightly intertwined and dynamically regulated to assure that only certain growth cones cross the midline, that those growth cones that cross do not linger at the midline, and that once they cross they never do so again.

Relevant Literature

Seeger, M., Tear, G., Ferres-Marco, D. and Goodman C.S. (1993) Neuron *10,* 409 - 426; Tear G., et al. (1996) Neuron *16*, 501 - 514; Rothberg et al. (1990) Genes Dev 4, 2169-2187; Kidd et al. (1998) Cell 92, 205-215.

SUMMARY OF THE INVENTION

The invention provides methods and compositions relating to vertebrate Slit1 and Slit2, collectively vertebrate Slit) polypeptides, related nucleic acids, polypeptide domains thereof having vertebrate Slit-specific structure and activity, and modulators of vertebrate Slit function. Vertebrate Slit polypeptides can regulate cell, especially nerve cell, function and morphology. The polypeptides may be produced recombinantly from transformed host cells from the subject vertebrate Slit polypeptide encoding nucleic acids or purified from mammalian cells. The invention provides isolated vertebrate Slit hybridization probes and primers capable of specifically hybridizing with natural vertebrate Slit genes, vertebrate Slit-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g. genetic hybridization screens for vertebrate Slit transcripts), therapy (e.g. to modulate nerve cell growth) and in the biopharmaceutical industry (e.g. as immunogens, reagents for isolating vertebrate Slit genes and polypeptides,

reagents for screening chemical libraries for lead pharmacological agents, etc.).

The invention also provides methods and compositions for identifying agents which modulate the interaction of Robo and a Robo ligand and for modulating the interaction of Robo and a Robo ligand. The methods for identifying Robo:ligand modulators find particular application in commercial drug screens. These methods generally comprise (1) combining a Robo polypeptide, a Slit polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, and (2) determining a second interaction of the Robo and Slit polypeptides in the presence of the agent, wherein a difference between the first and second interactions indicates that the aget modulates the interaction of the Robo and Slit polypeptides. The subject methods of modulating the interaction of Robo and a Robo ligand involve combining a Robo polypeptide, a Slit polypeptide and a modulator under conditions whereby, but for the presence of the modulator, the Robo and Slit polypeptides engage in a first interaction, whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction. In a particular embodiment, the modulator is dominant negative form of the Robo or Slit polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

The subject methods include screens for agents which modulate Robo:ligand interactions and methods for modulating Robo:ligand interactions. Robo activation is found to regulate a wide variety of cell functions, including cell-cell interactions, cell mobility, morphology, etc. Slit polypeptides are disclosed as specific activators and inactivators of Robo polypeptides. Accordingly, the invention provides methods for modulating targeted cell function comprising the step of modulating Robo activation by contacting the cell with a modulator of a Robo:Slit interaction..

The targeted Robo polypeptide is generally naturally expressed on the targeted cells. The nucleotide sequences of exemplary natural cDNAs encoding drosophila 1, drosophila 2, C. elegans, human 1, human 2 and mouse 1 Robo polypeptides and their translates are described in Kidd et al. (1998) Cell 92, 205-215 and USSN 08/971,172. The targeted Robo polypeptides comprise at least a functional Robo domain, which domain has Robo-specific amino acid sequence and binding specificity or function. Preferred Robo domains comprise

at least 8, preferably at least 16, more preferably at least 32, most preferably at least 64 consecutive residues of a natural full length Robo. In a particular embodiment, the domains comprise one or more structural/functional Robo immunoglobulin, fibronectin or cytoplasmic motif domains described herein. The subject domains provide Robo-specific antigens and/or immunogens, especially when coupled to carrier proteins. For example, peptides corresponding to Robo- and human Robo-specific domains are covalently coupled to keyhole limpet antigen (KLH) and the conjugate is emulsified in Freunds complete adjuvant. Laboratory rabbits are immunized according to conventional protocol and bled. The presence of Robo-specific antibodies is assayed by solid phase immunosorbant assays using immobilized Robo polypeptides. Generic Robo-specific peptides are readily apparent as conserved regions in aligned Robo polypeptide sequences. In addition, species-specific antigenic and/or immunogenic peptides are readily apparent as diverged extracellular or cytosolic regions in alignments Human Robo-specific antibodies are characterized as uncross-reactive with non-human Robo polypeptides.

The subject domains provide Robo domain specific activity or function, such as Robo-specific cell, especially neuron modulating or modulating inhibitory activity, Robo-ligand-binding or binding inhibitory activity. Robo-specific activity or function may be determined by convenient *in vitro,* cell-based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g. gene therapy, transgenics, etc.), etc. The binding target may be a natural intracellular binding target, a Robo regulating protein or other regulator that directly modulates Robo activity or its localization; or non-natural binding target such as a specific immune protein such as an antibody, or a Robo specific agent such as those identified in screening assays such as described below. Robo-binding specificity may be assayed by binding equilibrium constants (usually at least about $10^7 M^{-1}$, preferably at least about $10^8 M^{-1}$, more preferably at least about $10^9 M^{-1}$), by the ability of the subject polypeptide to function as negative mutants in Robo-expressing cells, to elicit Robo specific antibody in a heterologous host (e.g a rodent or rabbit), etc.

Similarly, the Slit polypeptide is conveniently selected from Slit polypeptides which specifically activate or inhibit the activation of the Robo polypeptide. Exemplary suitable Slit polypeptides (a) comprises a vertebrate Slit sequence disclosed herein, especially human Slit-1 (SEQ ID NO:02), or a deletion mutant thereof which specifically modulates Robo

expression or a sequence about 60-70%, preferably about 70-80%, more preferably about 80-90%, more preferably about 90-95%, most preferably about 95-99% similar to a vertebrate Slit sequence disclosed herein as determined by Best Fit analysis using default settings and is other than a natural drosophila Slit sequence, preferably other than a natural invertebrate Slit sequence, and/or (b) is encoded by a nucleic acid comprising a natural Slit encoding sequence (such as a natural human Slit-1 encoding sequence, SEQ ID NO:01) or a fragment thereof at least 36, preferably at least 72, more preferably at least 144, most preferably at least 288 nucleotides in length which specifically hybridizes thereto. Suitable deletion mutants are readily screened in Robo binding or activation assays as described herein. Preferred Slit domains/deletion mutants/fragemtns comprise at least 8, preferably at least 16, more preferably at least 32, most preferably at least 64 consecutive residues of a disclosed vertebrate Slit sequences and provide a Slit specific activity, such as Slit-specific antigenicity and/or immunogenicity, especially when coupled to carrier proteins as described above for Robo above. Suitable natural Slit encoding sequence fragements are of length sufficient to encode such Slit domains. In a particular embodiment, the Slit fragments comprise species specific fragments; such fragments are readily discerned from alignments of the disclosed sequences, see, e.g. shown as white backgrounded sequences in Tables 3 and 4. Exemplary such human Slit-1 immunogenic and/or antigenic peptides are shown in Table 1.

Table 1. Immunogenic human Slit-1 polypeptides eliciting Slit-1 specific rabbit polyclonal antibody: Slit polypeptide-KLH conjugates immunized per protocol described above.

Slit Polypeptide	Immunogenicity	Slit Polypeptide	Immunogenicity
SEQ ID NO:02, res. 1-10	+++	SEQ ID NO:02, res. 561-576	+++
SEQ ID NO:02, res. 29-41	+++	SEQ ID NO:02, res. 683-697	+++
SEQ ID NO:02, res. 75-87	+++	SEQ ID NO:02, res. 768-777	+++
SEQ ID NO:02, res. 92-109	+++	SEQ ID NO:02, res. 798-813	+++
SEQ ID NO:02, res. 132-141	+++	SEQ ID NO:02, res. 882-894	+++
SEQ ID NO:02, res. 192-205	+++	SEQ ID NO:02, res. 934-946	+++
SEQ ID NO:02, res. 258-269	+++	SEQ ID NO:02, res. 1054-1067	+++
SEQ ID NO:02, res. 295-311	+++	SEQ ID NO:02, res. 1181-1192	+++
SEQ ID NO:02, res. 316-330	+++	SEQ ID NO:02, res. 1273-1299	+++
SEQ ID NO:02, res. 373-382	+++	SEQ ID NO:02, res. 1383-1397	+++
SEQ ID NO:02, res. 403-422	+++	SEQ ID NO:02, res. 1468-1477	+++
SEQ ID NO:02, res. 474-485	+++	SEQ ID NO:02, res. 1508-1517	+++

The subject domains provide Slit domain specific activity or function, such as Slit-

specific cell, especially neuron modulating or modulating inhibitory activity, Slit-ligand-binding or binding inhibitory activity. Slit-specific activity or function may be determined by convenient *in vitro,* cell-based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g. gene therapy, transgenics, etc.), etc. The binding target may be a natural intracellular binding target, a Slit regulating protein or other regulator that directly modulates Slit activity or its localization; or non-natural binding target such as a specific immune protein such as an antibody, or a Slit specific agent such as those identified in screening assays such as described below. Slit-binding specificity may be assayed by binding equilibrium constants (usually at least about $10^7 M^{-1}$, preferably at least about $10^8 M^{-1}$, more preferably at least about $10^9 M^{-1}$), by the ability of the subject polypeptide to function as negative mutants in Slit-expressing cells, to elicit Slit specific antibody in a heterologous host (e.g a rodent or rabbit), etc.

In one embodiment, the Slit polypeptides are encoded by a nucleic acid comprising SEQ ID NO:01 or a fragment thereof which hybridizes with a full-length strand thereof, preferably under stringent conditions. Such nucleic acids comprise at least 36, preferably at least 72, more preferably at least 144 and most preferably at least 288 nucleotides of SEQ ID NO:01. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M $NaPO_4$, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE (Conditions I); preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C (Conditions II). Exemplary nucleic acids which hybridize with a strand of SEQ ID NO:01 are shown in Table 2.

Table 2. Exemplary nucleic acids which hybridize with a strand of SEQ ID NO:01 under Conditions I and/or II.

Slit Nucleic Acid	Hybridization	Slit Nucleic Acid	Hybridization
SEQ ID NO:01, nucl. 1-47	+	SEQ ID NO:01, nucl. 1258-1279	+
SEQ ID NO:01, nucl. 58-99	+	SEQ ID NO:01, nucl. 1375-1389	+
SEQ ID NO:01, nucl. 95-138	+	SEQ ID NO:01, nucl. 1581-1595	+
SEQ ID NO:01, nucl. 181-220	+	SEQ ID NO:01, nucl. 1621-1639	+
SEQ ID NO:01, nucl. 261-299	+	SEQ ID NO:01, nucl. 1744-1755	+
SEQ ID NO:01, nucl. 274-315	+	SEQ ID NO:01, nucl. 1951-1969	+
SEQ ID NO:01, nucl. 351-389	+	SEQ ID NO:01, nucl. 2150-2163	+
SEQ ID NO:01, nucl. 450-593	+	SEQ ID NO:01, nucl. 2524-2546	+
SEQ ID NO:01, nucl. 524-546	+	SEQ ID NO:01, nucl. 2761-2780	+
SEQ ID NO:01, nucl. 561-608	+	SEQ ID NO:01, nucl. 2989-2999	+
SEQ ID NO:01, nucl. 689-727	+	SEQ ID NO:01, nucl. 3108-3117	+
SEQ ID NO:01, nucl. 708-737	+	SEQ ID NO:01, nucl. 3338-3351	+
SEQ ID NO:01, nucl. 738-801	+	SEQ ID NO:01, nucl. 3505-3514	+
SEQ ID NO:01, nucl. 805-854	+	SEQ ID NO:01, nucl. 3855-3867	+
SEQ ID NO:01, nucl. 855-907	+	SEQ ID NO:01, nucl. 4010-4025	+
SEQ ID NO:01, nucl. 910-953	+	SEQ ID NO:01, nucl. 4207-4219	+
SEQ ID NO:01, nucl. 1007-1059	+	SEQ ID NO:01, nucl. 4333-4345	+
SEQ ID NO:01, nucl. 1147-1163	+	SEQ ID NO:01, nucl. 4521-4529	+

A wide variety of cell types express Robo polypeptides subject to regulation by the disclosed methods, including many neuronal cells, transformed cells, infected (e.g. virus) cells, etc. Ascertaining Robo binding or activation is readily effected by binding assays or cells function assays as disclosed herein or in the cited copending applications. Accordingly, indications for the subject methods encompass a wide variety of cell types and function, including axon outgrowth, tumor cell invasion or migration, etc. The target cell may reside in culture or in situ, i.e. within the natural host. For in situ applications, the compositions are added to a retained physiological fluid such as blood or synovial fluid. For CNS administration, a variety of techniques are available for promoting transfer of the therapeutic across the blood brain barrier including disruption by surgery or injection, drugs which transiently open adhesion contact between CNS vasculature endothelial cells, and compounds which facilitate translocation through such cells. Slit polypeptides may also be amenable to direct injection or infusion, topical, intratracheal/nasal administration e.g. through aerosol, intraocularly, or within/on implants e.g. fibers e.g. collagen, osmotic pumps, grafts comprising appropriately transformed cells, etc. A particular method of administration involves coating, embedding or derivatizing fibers, such as collagen fibers, protein polymers,

etc. with therapeutic polypeptides. Other useful approaches are described in Otto et al. (1989) J Neuroscience Research 22, 83-91 and Otto and Unsicker (1990) J Neuroscience 10, 1912-1921. Generally, the amount administered will be empirically determined, typically in the range of about 10 to 1000 μg/kg of the recipient and the concentration will generally be in the range of about 50 to 500 μg/ml in the dose administered. Other additives may be included, such as stabilizers, bactericides, etc. will be present in conventional amounts.

In one embodiment, the invention provides administering the subject Slit polypeptides in combination with a pharmaceutically acceptable excipient such as sterile saline or other medium, gelatin, an oil, etc. to form pharmaceutically acceptable compositions. The compositions and/or compounds may be administered alone or in combination with any convenient carrier, diluent, etc. and such administration may be provided in single or multiple dosages. Useful carriers include solid, semi-solid or liquid media including water and non-toxic organic solvents. In another embodiment, the invention provides the subject compounds in the form of a pro-drug, which can be metabolically converted to the subject compound by the recipient host. A wide variety of pro-drug formulations for polypeptide-based therapeutics are known in the art. The compositions may be provided in any convenient form including tablets, capsules, troches, powders, sprays, creams, etc. As such the compositions, in pharmaceutically acceptable dosage units or in bulk, may be incorporated into a wide variety of containers. For example, dosage units may be included in a variety of containers including capsules, pills, etc. The compositions may be advantageously combined and/or used in combination with other therapeutic or prophylactic agents, different from the subject compounds. In many instances, administration in conjunction with the subject compositions enhances the efficacy of such agents, see e.g. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9^{th} Ed., 1996, McGraw-Hill.

In another aspect, the invention provides methods of screening for agents which modulate Robo-Slit interactions. These methods generally involve forming a mixture of a Robo-expressing cell, a Slit polypeptide and a candidate agent, and determining the effect of the agent on the amount of Robo expressed by the cell. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and

human trials; for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development. Cell and animal based neural guidance/repulsion assays are described in detail in the experimental section below.

The amino acid sequences of the disclosed vertebrate Slit polypeptides are used to back-translate Slit polypeptide-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural Slit-encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc, Madison WI). Slit-encoding nucleic acids used in Slit-expression vectors and incorporated into recombinant host cells, e.g. for expression and screening, transgenic animals, e.g. for functional studies such as the efficacy of candidate drugs for disease associated with Slit-modulated cell function, etc.

The invention also provides nucleic acid hybridization probes and replication / amplification primers having a vertebrate Slit cDNA specific sequence comprising a fragment of a disclosed vertebrate cDNA sequence, and sufficient to effect specific hybridization thereto. Such primers or probes are at least 12, preferably at least 24, more preferably at least 36 and most preferably at least 96 nucleotides in length. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M $NaPO_4$, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C. Slit nucleic acids can also be distinguished using alignment algorithms, such as BLASTX (Altschul *et al.* (1990) Basic Local Alignment Search Tool, J Mol Biol 215, 403-410). In addition, the invention provides nucleic acids having a sequence about 60-70%, preferably about 70-80%, more preferably about 80-90%, more preferably about 90-95%, most preferably about 95-99% similar to a vertebrate Slit sequence disclosed herein as determined by Best Fit analysis using default settings and is other than a natural drosophila Slit sequence, preferably other than a natural invertebrate Slit sequence. In a particular embodiment, the Slit polynucleotide fragments comprise species specific

fragments; such fragments are readily discerned from alignments of the disclosed sequences.

The subject nucleic acids are of synthetic/non-natural sequences and/or are recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. The subject recombinant nucleic acids comprising the nucleotide sequence of disclosed vertebrate Slit nucleic acids, or fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by (i.e. contiguous with) a sequence other than that which it is joined to on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, more preferably fewer than 500 bp, which is at a terminus or is immediately flanked by a sequence other than that which it is joined to on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

The subject nucleic acids find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.; use in detecting the presence of Slit genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional Slit homologs and structural analogs. In diagnosis, Slit hybridization probes find use in identifying wild-type and mutant Slit alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. In therapy, therapeutic Slit nucleic acids are used to modulate cellular expression or intracellular concentration or availability of active Slit. Exemplary human Slit-1 probes and primers are shown in Table 5 (A and B) and Table 6.

The following examplary assay is offered by way of illustration and not by way of limitation:

EXAMPLES

Protocol for Ligand Screening of Transfected COS cells.

I. Prepare the Ligand

Expression Construct: cDNAs encoding targeted Slit polypeptides are tagged with the Fc portion of human IgG and subcloned into a 293 expression vector (pCEP4: In Vitrogen).

Transfection: 293 EBNA cells are transfected ($CaPO_4$ method) with the Slit

expression constructs. After 24 h recovery, transfected cells are selected with G418 (geneticin, 250 ug/ml, Gibco) and hygromycin (200 ug/ml). Once the selection process is complete, cells are maintained in Dulbecco's Modified Eagles medium (DME)/10% FCS under selection.

Preparation of Conditioned Medium: Serum-containing media is replaced with Optimem with glutamax-1 (Gibco) and 300 ng/ml heparin (Sigma), and the cells are conditioned for 3 days. The media is collected and spun at 3,000xg for 10 minutes. The supernatant is filtered (0.45 um) and stored with 0.1% azide at 4°C for no more than 2 weeks.

II. Prepare Truncated Receptor (Positive Control)

Expression Construct: cDNA encoding a corresponding Robo C-terminal deletion mutant comprising the extracellular domain (truncated immediately N-terminal to the transmembrane region) is subcloned into a 293 expression vector (pCEP4: In Vitrogen).

Transfection: 293 EBNA cells are transfected ($CaPO_4$ method) with the receptor mutant expression construct. After 24 h recovery, transfected cells are selected with G418 (geneticin, 250 ug/ml, Gibco) and hygromycin (200 ug/ml). Once the selection process is complete, cells are maintained in Dulbecco's Modified Eagles medium (DME)/10% FCS under selection.

Preparation of Conditioned Medium: Serum-containing media is replaced with Optimem with glutamax-1 (Gibco) and 300 ng/ml heparin (Sigma), and the cells are conditioned for 3 days. The media is collected and spun at 3,000xg for 10 minutes. The supernatant is filtered (0.45 um) and stored with 0.1% azide at 4°C for no more than 2 weeks.

II. Transfect COS Cells

Seed COS cells (250,000) on 35 mm dishes in 2 ml DME/10% FCS.

18-24 h later, dilute 1 ug of Robo-encoding DNA (cDNA cloned into pMT21 expression vector) into 200 ul serum-free media and add 6 ul of Lipofectamine (Gibco). Incubate this solution at room temperature for 15-45 min.

Wash the cells 2X with PBS. Add 800 ul serum-free media to the tube containing the lipid-DNA complexes. Overlay this solution onto the washed cells.

Incubate for 6 h. Stop the reaction by adding 1 ml DMA/20% FCS. Refeed cells. Assay cells 12 hr later.

III. Ligand Binding Assay

Wash plates of transfected COS cells 1X with cold PBS (plus Ca/Mg)/1% goat serum. Add 1 ml conditioned media neat and incubate 90 min at room temp.

Wash plates 3X with PBS (plus Ca/Mg). On the 4th wash, add 1 ml 50% methanol to 1 ml PBS. Then add 1 ml methanol. Evacuate and add 1 ml methanol.

Wash 1X with PBS. Wash 1X PBS/1% goat serum.

Add secondary antibody (1-to-2,000 anti-human Fc conjugated to alkaline phosphatase (Jackson Lab)) in PBS/1% goat serum. Incubate 30-40 min room temp.

Wash 3X with PBS. Wash 1X alkaline phosphatase buffer (100 mM Tris-Cl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$). Prepare alkaline phosphatase reagents: 4.5 ul/ml NBT and 3.5 ul/ml BCIP (Gibco) in alkaline phosphatase buffer.

Incubate 10-30 min, quench with 20 mM EDTA in PBS. Cells that have bound Slit polypeptides are visible by the presence of a dark purple reaction product.

In parallel incubations, positive controls are provided by titrating Slit binding with serial dilutions of the mutant receptor conditioned medium.

IV. Results: Binding of Slit to Robo

Cell expressing mammalian Slit polypeptides were shown to bind Robo. No reactivity was observed with control COS cells or with receptor-expressing COS cells in the presence of the secondary antibody but in the absence of the Slit-Fc fusion. Binding was observed to receptor-expression cells using a construct in which a Slit polypeptide is fused directly to alkaline phosphatase, for which a secondary antibody is not required. Receptor deletion mutants titrate the Slit-Robo binding, serving as a positive control for inhibition assays.

Protocol for high throughput Robo-Slit binding assay.

A. Reagents:

- Neutralite Avidin: 20 μg/ml in PBS.

- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.

- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 1 mM $MgCl_2$, 1% glycerol, 0.5% NP-40, 50 mM β-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors.

- ^{33}P Robo polypeptide 10x stock: 10^{-8} - 10^{-6} M "cold" Robo polypeptide specific Robo domain supplemented with 200,000-250,000 cpm of labeled Robo (Beckman counter). Place in the 4°C microfridge during screening.

- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2mM $NaVO_3$ (Sigma # S-6508) in 10 ml of PBS.

-Slit: 10^{-7} - 10^{-5} M biotinylated Slit in PBS.

B. Preparation of assay plates:

- Coat with 120 µl of stock N-Avidin per well overnight at 4°C.
- Wash 2 times with 200 µl PBS.
- Block with 150 µl of blocking buffer.
- Wash 2 times with 200 µl PBS.

C. Assay:

- Add 40 µl assay buffer/well.
- Add 10 µl compound or extract.
- Add 10 µl ^{33}P-Robo (20-25,000 cpm/0.1-10 pmoles/well =10^{-9}- 10^{-7} M final conc).
- Shake at 25°C for 15 minutes.
- Incubate additional 45 minutes at 25°C.
- Add 40 µM biotinylated Slit (0.1-10 pmoles/40 ul in assay buffer)
- Incubate 1 hour at room temperature.
- Stop the reaction by washing 4 times with 200 µM PBS.
- Add 150 µM scintillation cocktail.
- Count in Topcount.

D. Controls for all assays (located on each plate):

a. Non-specific binding

b. Soluble (non-biotinylated Slit) at 80% inhibition.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of identifying agents which modulate the interaction of Robo and a Robo ligand, said method comprising the steps of:

combining a Robo polypeptide, a Slit polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, wherein the Slit polypeptide specifically binds, activates or inhibits the activation of the Robo polypeptide and

determining a second interaction of the Robo and Slit polypeptides in the presence of the agent,

wherein a difference between the first and second interactions indicates that the aget modulates the interaction of the Robo and Slit polypeptides.

2. A method of modulating the interaction of Robo and a Robo ligand, said method comprising the step of

combining a Robo polypeptide, a Slit polypeptide and a modulator under conditions whereby, but for the presence of the modulator, the Robo and Slit polypeptides engage in a first interaction, wherein the Slit polypeptide specifically binds, activates or inhibits the activation of the Robo polypeptide and

whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction.

3. A method according to claim 2, wherein the modulator is a dominant negative form of the Robo or Slit polypeptide.

4. An isolated Slit polypeptide comprising a vertebrate species-specific Slit fragment.

5. An isolated vertebrate Slit polypeptide according to claim 4, wherein said vertebrate is human, mouse or rat.

6. A recombinant nucleic acid encoding a vertebrate Slit polypeptide according to claim 4.

7. A recombinant Slit nucleic acid comprising a strand of SEQ ID NO:01, or a fragment thereof having at least 24 consecutive nucleotides thereof, and sufficient to specifically hybridize with a polynucleotide having the sequence defined by the corresponding opposite strand of SEQ ID NO:01, and is other than a natural drosophila Slit sequence.

ABSTRACT OF THE DISCLOSURE

Disclosed are methods and compositions for identifying agents which modulate the interaction of Robo and a Robo ligand and for modulating the interaction of Robo and a Robo ligand. The methods for identifying Robo:ligand modulators find particular application in commercial drug screens. These methods generally comprise (1) combining a Robo polypeptide, a Slit polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, and (2) determining a second interaction of the Robo and Slit polypeptides in the presence of the agent, wherein a difference between the first and second interactions indicates that the aget modulates the interaction of the Robo and Slit polypeptides. The subject methods of modulating the interaction of Robo and a Robo ligand involve combining a Robo polypeptide, a Slit polypeptide and a modulator under conditions whereby, but for the presence of the modulator, the Robo and Slit polypeptides engage in a first interaction, whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction. In a particular embodiment, the modulator is dominant negative form of the Robo or Slit polypeptide.

Table 1.

Alignment of human Slit-1 (SEQ ID NO:02), human Slit-2 (SEQ ID NOS:03-06), Drosophila Slit-1 (SEQ ID NO:07), C. elegans Slit-1 (SEQ ID NOS:08-09), mouse Slit-2 (SEQ ID NOS:10-11) and mouse Slit-1 (SEQ ID NOS:12-14).

```
1    M A A P S R T T L M P P P F R L Q L R L - L I L P I L L L L R H D A V H A E P Y D-Slit
1    M R G V G W Q - - - - - - - - M L S L S L G L V L A I L - - - - - - - - - - - - H-Slit1

40   S G G F G S S A V S S G G L G S V G I H I P G G G V G V I T E A R C P R V C S C D-Slit
21   - - - - - - - - - - - - - - - - - - - - - - - - - - N K V A P Q A C P A Q C S C H-Slit1

80   T G L N V D C S H R G L T S V P R K I S A D V E R L E L Q G N N L T V I Y E T D D-Slit
35   S G S T V D C H G L A L R S V P R N I P R N T E R L D L N G N N I T R I T K T D H-Slit1

120  F Q R L T K L R M L Q L T D N Q I H T I E R N S F Q D L V S L E R L - - - - - - D-Slit
75   F A G L R H L R V L Q L M E N K I S T I E R G A F Q D L K E L E R L R L N R N H H-Slit1
1    - - - - - H L R V L Q L M E N R I S T I E R G A F Q D L K E L E R L R L N R N N M-Slit1

154  - - - - - - - - - - - - - - - - - - D I S N N V I T T V G R R V F K G A Q S L R D-Slit
115  L Q L F P E L L F L G T A K L Y R L D L S E N Q I Q A I P R K A F R G A V D I K H-Slit1
36   L Q L F P E L L F L G T A R L Y R L D L S E N Q I Q A I P R K A F R G A V D I K M-Slit1

176  S L Q L D N N Q I T C L D E H A F K G L V E L E I L T L N N N N L T S L P H N I D-Slit
155  N L Q L D Y N Q I S C I E D G A F R A L R D L E V L T L N N N N I T R L S V A S H-Slit1
76   N L Q L D Y N Q I S C I E D G A F R A L R D L E V L T L N N N N I T R L S V A S M-Slit1

216  F G G L G R L R A L R L S D N P F A C D C H L S W L S R F L R S A T R L A P Y T D-Slit
195  F N H M P K L R T F R L H S N N L Y C D C H L A W L S D W L R K R P R V G L Y T H-Slit1
116  F N H M P K L R T F R L H S N N L Y C                                           M-Slit1

256  R C Q S P S Q L K G Q N V A D L H D Q E F K C S G L T E - H A P M - - - E C G A D-Slit
235  Q C M G P S H L R G H N V A E V Q K R E F V C S D E E E G H Q S F M A P S C S V H-Slit1

292  E N S C P H P C R C A D G I V D C R E K S L T S V P V T L P D D T T D V R L E Q D-Slit
275  L H - C P A A C T C S N N I V D C R G K G L T E I P T N L P E T I T E I R L E Q H-Slit1
1    - - - - - S P C T C S N N I V D C R G K G L M E I P A N L P E G I V E I R L E Q H-Slit2

332  N F I T E L P P K S F S S F R R L R R I D L S N N N I S R I A H D A L S G L K Q D-Slit
314  N T I K V I P P G A F S P Y K K L R R I D L S N N Q I S E L A P D A F Q G L R S H-Slit1
36   N S I K A I P A G A F T Q Y K K L K R I D I S K N Q I S D I A P D A F Q G L K S H-Slit2

372  L T T L V L Y G N K I K D L P S G V F K G L G S L R L L L L N A N E I S C I R K D-Slit
354  L N S L V L Y G N K I T E L P K S L F E G L F S L Q L L L L N A N K I N C L R V H-Slit1
76   L T S L V L Y G N K I T E I A K G L F D G L V S L Q L L L L                     H-Slit2

1                                                                                R CE-Slit
412  D A F R D L H S L S L L S L Y D N N I Q S L A N G T F D A M K S M K T V H L A K D-Slit
394  D A F Q D L H N L N L L S L Y D N K L Q T I A K G T F S P L R A I Q T M H L A Q H-Slit1
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2    N P X I C D C N L Q W L A Q I N L Q K N I E T S G A R C E Q P K R L R K K K F A CE-Slit
452  N P F I C D C N L R W L A D Y L H K N P I E T S G A R C E S P K R M H R R R I E D-Slit
434  N P F I C D C H L K W L A D Y L H T N P I E T S G A R C T S P R R L A N K R I G H-Slit1

42   T L P P N K F K C K G S E S F V S M Y A D S C F I D S I C P T Q C D C Y G T T V CE-Slit
492  S L R E E K F K C S - W G E L R M K L S G E C R M D S D C P A M C H C E G T T V D-Slit
474  Q I K S K K F R C S G T E D Y R S K L S G D C F A D L A C P E K C R C E G T T V H-Slit1

82   D C N K R G L N T I P T S I P R F A T Q L L L S G N N I S T V D L N S N I H V L CE-Slit
531  D C T G R R L K E I P R D I P L H T T E L L L N D N E L G R I S S D G L F G R L D-Slit
514  D C S N Q K L N K I P E H I P Q Y T A E L R L N N N E F T V L E A T G I F K K L H-Slit1

122  E N L E X L D L S N N H I T F I N D K S F E K L S K L R E L X L N D - - - - - - CE-Slit
571  P H L V K L E L K R N Q L T G I E P N A F E G A S H I Q E L Q L G E N K I K E I D-Slit
554  P Q L R K I N F S N N K I T D I E E G A F E G A S G V N E I L L T S N R L E N V H-Slit1
1    - - - - - - - - - - - - - - - - - E G A F N G A A S V Q E L M L T G N Q L E T V H-Slit2

611  S N K M F - - - - - - - - - - - - - - - - - - - - - - - - - L G L H Q L K T L N D-Slit
594  Q H K M F K G - L E S L K T L M L R S N R I T C V G N D S F I G L S S V R L L S H-Slit1
24   H G R G F R G G L S G L K T L M L R S N L I G C V S N D T F A G L S S V R L L S H-Slit2

626  L Y D N Q I S C V M P G S F E H L N S L T S L N L A S N P F N C N C H L A W - F D-Slit
633  L Y D N Q I T T V A P G A F D T L H S L S T L N L L A N P F N C N C Y L A W - L H-Slit1
64   L Y D N R I T T I T P G A F T T L V S L S T I N L L S N P F N C N C H L G A G L H-Slit2

665  A E C V R K K S L N G G A A R C G A P S K V R D V Q I K D L P H S E F K C S S E D-Slit
672  G E W L R K K R I V T G N P R C Q K P Y F L K E I P I Q D V A I Q D F T C D D G H-Slit1
104  G K W L R K R R I V S G N P R C Q K P F F L K E I P I Q G V G H P G I           H-Slit2

1                                                              S N K N L T S F P S R I P F D CE-Slit
705  N S E - G C L G D G Y C P P S C T C T G T V V A C S R N Q L K E I P R G I P A E D-Slit
712  N D D N S C S P L S R C P T E C T C L D T V V R C S N K G L K V L P K G I P R D H-Slit1

16   T T E L Y L D A N Y I N E I P A H D L N R L Y S L T K L D L S H N R L I S L E N CE-Slit
744  T S E L Y L E S N E I E Q I H Y E R I R H L R S L T R L D L S N N Q I T I L S N D-Slit
752  V T E L Y L D G N Q F T L V P K E - L S N Y K H L T L I D L S N N R I S T L S N H-Slit1

56   N T F S N L T R L S T L I I S Y N K L R C L Q P L A F N G L N A L R I L S L H G CE-Slit
784  Y T F A N L T K L S T L I I S Y N K L Q C L Q R H A L S G L N N L R V V S L H G D-Slit
791  Q S F S N M T Q L L T L I L S Y N R L R C I P P R T F D G L K S L R L L S L H G H-Slit1

96   N D I S F L P Q S A F S N L T S I T H I A V G S N S L Y C D C N M A W F S K W I CE-Slit
824  N R I S M L P E G S F E D L K S L T H I A L G S N P L Y C D C G L K W F S D W I D-Slit
831  N D I S V V P E G A F N D L S A L S H L A I G A N P L Y C D C N M Q W L S D W V H-Slit1

136  K S K F I E A G I A R C E Y P N T V S N Q L L L T A Q P Y Q F T C D S K V P T K CE-Slit
864  K L D Y V E P G I A R C A E P E Q M K D K L I L S T P S S S F V C R G R V R N D D-Slit
871  K S E Y K E P G I A R C A G P G E M A D K L L L T T P S K K F T C Q G P V D V N H-Slit1
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176  LATKCDLCLNSPCKNNAICETTSSRKYTCNCTPGFYGVHC  CE-Slit
904  ILAKCNACFEQPCQNQAQCVALPQREYQCLCQPGYHGKHC  D-Slit
911  ILAKCNPCLSNPCKNDGTCNSDPVDFYRCTCPYGFKGQDC  H-Slit1

216  ENQIDACYGSPCLNNATCKV--AQAGRFNCYCNKGFEGDY  CE-Slit
944  EFMIDACYGNPCRNNATCTV--LEEGRFSCQCAPGYTGAR  D-Slit
951  DVPIHACISNPCKHGGTCHLKEGEEDGFWCICADGFEGEN  H-Slit1

254  CEKNIDDCVNS-KCENGGKCVDLVRFCSEELKNFQSFQIN  CE-Slit
982  CETNIDDCLGEIKCQNNATCID---------------GVE  D-Slit
991  CEVNVDDC-EDNDCENNSTCVD---------------GIN  H-Slit1

293  SYRCDCPMEYEGKHCEDKLEYCTKKLNPCENNGKCIPING  CE-Slit
1007 SYKCECQPGFSGEFCDTKIQFCSPEFNPCANGAKCMDHFT  D-Slit
1015 NYTCLCPPEYTGELCEEKLDFCAQDLNPCQHDSKCILTPK  H-Slit1
                                         DPLPV  M-Slit2

333  SYSCMCSPGFTGNNCETNIDDCKNVECQNGGSCVDGILSY  CE-Slit
1047 HYSCDCQAGFHGTNCTDNIDDCQNHMCQNGGTCVDGINDY  D-Slit
1055 GFKCDCTPGYVGEHCDIDFDDCQDNKCKNGAHCTDAVNGY  H-Slit1
1    -----------------NNDDCVGHKCRHGAQCVDEVNGY  M-Slit1
1    WPRCECMPGYAGDNCSENQDDCRDHRCQNGAQCMDEVNSY  H-Slit2
6    HHRCECMLGYTGDNCSENQDDCKDHKCQNGAQCVDEVNSY  M-Slit2

373  DCLCRPGYAGQYCEIPPMMDMEYQKTDACQQSACGQG-EC  CE-Slit
1087 QCRCPDDYTGKYCEGHNMISMMYPQTSPCQNHECKHGV-C  D-Slit
1095 TCICPEGYSGLFCEFSP--PMVLPRTSPCDNFDCQNGAQC  H-Slit1
24   TCICPQGFSGLFCEHPP--PMVLLQTSPCDQYECQNGAQC  M-Slit1
41   SCLCAEGYSGQLCEIPP--HLPAPK-SPCEGTECQNGANC  H-Slit2
46   ACLCVEGYSGQLCEIPP-----APR-SSCEGTECQNGANC  M-Slit2

412  VASQN-SSDFTCKCHEGFSGPSCDRQMSVGFKNPGAYLAL  CE-Slit
1126 FQPNAQGSDYLCRCHPGYTGKWCEYLTSISFVHNNSFVEL  D-Slit
1133 IVRINEP---ICQCLPGYQGEKCEKLVSVNFINKESYLQI  H-Slit1
62   IVVQQEP---TCRCPPGFAGPRCEKLITVNFVGKDSYVEL  M-Slit1
78   VDQGNRP---VCQCLPGFGGPECEKLLSVNFVDRDTYLQF  H-Slit2
80   VDQGSRP---VCQCLPGFGGPECEKLLSVNFVDRDTYLQF  M-Slit2

451  DPLAS--DGTITMTLRTTSKIGILLYYGDDHFVSAELYDG  CE-Slit
1166 EPLRTRPEANVTIVFSSAEQNGILMYDGQDAHLAVELFNG  D-Slit
1170 PSAKVRPQTNITLQIATDEDSGILLYKGDKDHIAVELYRG  H-Slit1
99   ASAKVR                                    M-Slit1
115  TDLQNWXRXNITLQVFTAEDNGILLYNGGNDHIAVXLYXG  H-Slit2
117  TDLQNWPRANITLQVSTAEDNGILLYNGDNDHIAVELY    M-Slit2

489  RVKLVYYIGNFPASHMYSSVKVNDGLPHRISIRTSERKCF  CE-Slit
1206 RIRVSYDVGNHPVSTMYSFEMVADGKYHAVELLAIKKNFT  D-Slit
1210 RVRASYDTGSHPASAIYSVETINDGNFHIVELLALDQSLS  H-Slit1
155  HVRFSY                                    H-Slit2

529  LQIDKNPVQIVENSGKSDQLITKGKEMLYIGGLPIEKSQD  CE-Slit
1246 LRVDRGLARSIINEGSNDYL--KLTTPMFLGGLPVDPAQQ  D-Slit
1250 LSVDGGNPKIITNLSKQSTL--NFDSPLYVGGMPGKSNVA  H-Slit1
1    IL-----------------------------------DVA  M-Slit1
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569   A K R R F H V K N S E S L K G C I S S I T I N E V P I N L Q Q A L E N V N T E Q   CE-Slit
1284  A Y K N W Q I R N L T S F K G C M K E V W I N H K L V D F G N A Q R Q Q K I T P   D-Slit
1288  S L R Q A P G Q N G T S F H G C I R N L Y I N S E L Q D F Q K V P M Q T G I L P   H-Slit1
6     S L R Q A P G E N G T S F H G C I R N L Y I N S E L Q D F R K M P M Q T G I L P   M-Slit1

609   S C - - - - - - - - - - - - - - - S A T V N F - - - - - - - - - - - - - - - - -   CE-Slit
1324  G C A L - - - - L E G E Q Q E E E D D E Q D F M D E - - - - - - T P H I K E E P   D-Slit
1328  G C E P C H K K V C A H G T C Q P S S Q A G F T C E C Q E G W M G P L C D Q R T   H-Slit1
46    G C E P C H K K V C A H G C C Q P S S Q S G F T C E C E E G W M G P L C D Q R T   M-Slit1

617   - - - C A G I D C G N G - K C T N N A L S P K G Y M C Q C D S H F S G E H C D E   CE-Slit
1354  V D P C L E N K C R R G S R C V P N S N A R D G Y Q C K C K H G Q R G R Y C D Q   D-Slit
1368  N D P C L G N K C V H G T - C L P I N A F - - S Y S C K C L E G H G G V L C D E   H-Slit1
86    N D P C L G N K C V H G T - C L P I N A F - - S Y S C K C L E G H G G V L C D E   M-Slit1

653   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   CE-Slit
1394  G E G S T E P - - - - - - - - - - - - - - - - - - - - - - - - P T V T A A S - -   D-Slit
1405  E E D L F N P C Q A I K C K H G K C R L S G L G Q P Y C E C S S G Y T G D S C D   H-Slit1
123   E E D L F N P C Q M I K C K H G K C R L S G V G Q P Y C E C N S G F T G D S C D   M-Slit1
1     - - - - - - - - - - - - - - - - Q C H I S D Q G E P Y C L C Q P G F S G E H C Q   H-Slit2
1     - - - - - - - - - A F K C H H G Q C H I S D R G E P Y C L C Q P G F S G H H C E   M-Slit2

655   K R I K C D K Q K F R R H H I E N E - - - - C R S V D R I K I A E C N G Y C G G   CE-Slit
1405  T - - - C R K E Q V R E Y Y T E N D - - - - C R S R Q P L K Y A K C V G G C G -   D-Slit
1445  R E I S C R G E R I R D Y Y Q K Q Q G Y A A C Q T T K K V S R L E C R G G C A G   H-Slit1
163   R E I S C R G E R I R D Y Y Q K Q Q G Y A A C Q T T K K V S R L E C R G G C A G   M-Slit1
25    Q E N P C L G Q V V R E V I R R Q K G Y A S C A T A S K V P I M E C R G G C - G   H-Slit2
32    Q E N P C M G E I V R E A I R R Q K D Y A S C A T A S K V P I M E C R G G C - G   M-Slit2

689   E Q N C C T A V K K K Q R K V K M I C K N G T T K I S T V H I I R Q C Q C E P T   CE-Slit
1440  - N Q C C A A K I V R R R K V R M V C S N N R K Y I K N L D I V R K C G C - - T   D-Slit
1485  G Q - C C G P L R S K R R K Y S F E C T D G S S F V D E V E K V V K C G C T R -   H-Slit1
203   G Q - C C G P L R S K R R K Y S F E C T D G S S F V D E V E K V V K C G C A R -   M-Slit1
64    P Q - C C Q P T R S K R R K Y V F Q C T D G S S F V E E V E R H L E C G C L A -   H-Slit2
71    T T - C C Q P I R S K R R K Y V F Q C T D G S S F V E E V E R H L E C G C R A -   M-Slit2

729   K S V L - - S E K                                                                 CE-Slit
1477  K K C Y                                                                           D-Slit
1523  - - - - C V S                                                                     H-Slit1
241   - - - - C A S                                                                     M-Slit1
102   - - - - C - S                                                                     H-Slit2
109   - - - - C - S                                                                     M-Slit2
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Table 2.

Alignment of human Slit-1 (SEQ ID NO:02) and Drosophila Slit-1 (SEQ ID NO:07)

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1    M A A P S R T T L M P P P F R L Q L R L - L I L P I L L L L R H D A V H A E P Y  D-Slit
1    M R G V G W Q - - - - - - - - M L S L S L G L V L A I L - - - - - - - - - - - -  H-Slit1

40   S G G F G S S A V S S G G L G S V G I H I P G G G V G V I T E A R C P R V C S C  D-Slit
21   - - - - - - - - - - - - - - - - - - - - - - - - - - N K V A P Q A C P A Q C S C  H-Slit1

80   T G L N V D C S H R G L T S V P R K I S A D V E R L E L Q G N N L T V I Y E T D  D-Slit
35   S G S T V D C H G L A L R S V P R N I P R N T E R L D L N G N N I T R I T K T D  H-Slit1

120  F Q R L T K L R M L Q L T D N Q I H T I E R N S F Q D L V S L E R L - - - - - -  D-Slit
75   F A G L R H L R V L Q L M E N K I S T I E R G A F Q D L K E L E R L R L N R N H  H-Slit1

154  - - - - - - - - - - - - - - - - - - D I S N N V I T T V G R R V F K G A Q S L R  D-Slit
115  L Q L F P E L L F L G T A K L Y R L D L S E N Q I Q A I P R K A F R G A V D I K  H-Slit1

176  S L Q L D N N Q I T C L D E H A F K G L V E L E I L T L N N N N L T S L P H N I  D-Slit
155  N L Q L D Y N Q I S C I E D G A F R A L R D L E V L T L N N N N I T R L S V A S  H-Slit1

216  F G G L G R L R A L R L S D N P F A C D C H L S W L S R F L R S A T R L A P Y T  D-Slit
195  F N H M P K L R T F R L H S N N L Y C D C H L A W L S D W L R K R P R V G L Y T  H-Slit1

256  R C Q S P S Q L K G Q N V A D L H D Q E F K C S G L T E - H A P M - - - E C G A  D-Slit
235  Q C M G P S H L R G H N V A E V Q K R E F V C S D E E E G H Q S F M A P S C S V  H-Slit1

292  E N S C P H P C R C A D G I V D C R E K S L T S V P V T L P D D T T D V R L E Q  D-Slit
275  L H - C P A A C T C S N N I V D C R G K G L T E I P T N L P E T I T E I R L E Q  H-Slit1

332  N F I T E L P P K S F S S F R R L R R I D L S N N N I S R I A H D A L S G L K Q  D-Slit
314  N T I K V I P P G A F S P Y K K L R R I D L S N N Q I S E L A P D A F Q G L R S  H-Slit1

372  L T T L V L Y G N K I K D L P S G V F K G L G S L R L L L L N A N E I S C I R K  D-Slit
354  L N S L V L Y G N K I T E L P K S L F E G L F S L Q L L L L N A N K I N C L R V  H-Slit1

412  D A F R D L H S L S L L S L Y D N N I Q S L A N G T F D A M K S M K T V H L A K  D-Slit
394  D A F Q D L H N L N L L S L Y D N K L Q T I A K G T F S P L R A I Q T M H L A Q  H-Slit1

452  N P F I C D C N L R W L A D Y L H K N P I E T S G A R C E S P K R M H R R R I E  D-Slit
434  N P F I C D C H L K W L A D Y L H T N P I E T S G A R C T S P R R L A N K R I G  H-Slit1

492  S L R E E K F K C S - W G E L R M K L S G E C R M D S D C P A M C H C E G T T V  D-Slit
474  Q I K S K K F R C S G T E D Y R S K L S G D C F A D L A C P E K C R C E G T T V  H-Slit1

531  D C T G R R L K E I P R D I P L H T T E L L L N D N E L G R I S S D G L F G R L  D-Slit
514  D C S N Q K L N K I P E H I P Q Y T A E L R L N N N E F T V L E A T G I F K K L  H-Slit1
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571  P H L V K L E L K R N Q L T G I E P N A F E G A S H I Q E L Q L G E N K I K E I D-Slit
554  P Q L R K I N F S N N K I T D I E E G A F E G A S G V N E I L L T S N R L E N V H-Slit1

611  S N K M F L G L H Q L K T L - - - - - - - - - - - - - - - - - - - - - - - - - N L D-Slit
594  Q H K M F K G L E S L K T L M L R S N R I T C V G N D S F I G L S S V R L L S L H-Slit1

627  Y D N Q I S C V M P G S F E H L N S L T S L N L A S N P F N C N C H L A W F A E D-Slit
634  Y D N Q I T T V A P G A F D T L H S L S T L N L L A N P F N C N C Y L A W L G E H-Slit1

667  C V R K K S L N G G A A R C G A P S K V R D V Q I K D L P H S E F K C S S E N S D-Slit
674  W L R K K R I V T G N P R C Q K P Y F L K E I P I Q D V A I Q D F T C D D G N D H-Slit1

707  E - G C L G D G Y C P P S C T C T G T V V A C S R N Q L K E I P R G I P A E T S D-Slit
714  D N S C S P L S R C P T E C T C L D T V V R C S N K G L K V L P K G I P R D V T H-Slit1

746  E L Y L E S N E I E Q I H Y E R I R H L R S L T R L D L S N N Q I T I L S N Y T D-Slit
754  E L Y L D G N Q F T L V P K E - L S N Y K H L T L I D L S N N R I S T L S N Q S H-Slit1

786  F A N L T K L S T L I I S Y N K L Q C L Q R H A L S G L N N L R V V S L H G N R D-Slit
793  F S N M T Q L L T L I L S Y N R L R C I P P R T F D G L K S L R L L S L H G N D H-Slit1

826  I S M L P E G S F E D L K S L T H I A L G S N P L Y C D C G L K W F S D W I K L D-Slit
833  I S V V P E G A F N D L S A L S H L A I G A N P L Y C D C N M Q W L S D W V K S H-Slit1

866  D Y V E P G I A R C A E P E Q M K D K L I L S T P S S S F V C R G R V R N D I L D-Slit
873  E Y K E P G I A R C A G P G E M A D K L L L T T P S K K F T C Q G P V D V N I L H-Slit1

906  A K C N A C F E Q P C Q N Q A Q C V A L P Q R E Y Q C L C Q P G Y H G K H C E F D-Slit
913  A K C N P C L S N P C K N D G T C N S D P V D F Y R C T C P Y G F K G Q D C D V H-Slit1

946  M I D A C Y G N P C R N N A T C T V L E - - E G R F S C Q C A P G Y T G A R C E D-Slit
953  P I H A C I S N P C K H G G T C H L K E G E E D G F W C I C A D G F E G E N C E H-Slit1

984  T N I D D C L G E I K C Q N N A T C I D G V E S Y K C E C Q P G F S G E F C D T D-Slit
993  V N V D D C - E D N D C E N N S T C V D G I N N Y T C L C P P E Y T G E L C E E H-Slit1

1024 K I Q F C S P E F N P C A N G A K C M D H F T H Y S C D C Q A G F H G T N C T D D-Slit
1032 K L D F C A Q D L N P C Q H D S K C I L T P K G F K C D C T P G Y V G E H C D I H-Slit1

1064 N I D D C Q N H M C Q N G G T C V D G I N D Y Q C R C P D D Y T G K Y C E G H N D-Slit
1072 D F D D C Q D N K C K N G A H C T D A V N G Y T C I C P E G Y S G L F C E F S P H-Slit1

1104 M I S M M Y P Q T S P C Q N H E C K H G V - C F Q P N A Q G S D Y L C R C H P G D-Slit
1112 - - P M V L P R T S P C D N F D C Q N G A Q C I - - - V R I N E P I C Q C L P G H-Slit1

1143 Y T G K W C E Y L T S I S F V H N N S F V E L E P L R T R P E A N V T I V F S S D-Slit
1147 Y Q G E K C E K L V S V N F I N K E S Y L Q I P S A K V R P Q T N I T L Q I A T H-Slit1
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1183 A E Q N G I L M Y D G Q D A H L A V E L F N G R I R V S Y D V G N H P V S T M Y D-Slit
1187 D E D S G I L L Y K G D K D H I A V E L Y R G R V R A S Y D T G S H P A S A I Y H-Slit1

1223 S F E M V A D G K Y H A V E L L A I K K N F T L R V D R G L A R S I I N E G S N D-Slit
1227 S V E T I N D G N F H I V E L L A L D Q S L S L S V D G G N P K I I T N L S K Q H-Slit1

1263 D Y L K L T T P M F L G G L P V D P A Q Q A Y K N W Q I R N L T S F K G C M K E D-Slit
1267 S T L N F D S P L Y V G G M P G K S N V A S L R Q A P G Q N G T S F H G C I R N H-Slit1

1303 V W I N H K L V D F G N A Q R Q Q K I T P G C A L - - - - L E G E Q Q E E E D D D-Slit
1307 L Y I N S E L Q D F Q K V P M Q T G I L P G C E P C H K K V C A H G T C Q P S S H-Slit1

1339 E Q D F M D E - - - - - - - T P H I K E E P V D P C L E N K C R R G S R C V P N S D-Slit
1347 Q A G F T C E C Q E G W M G P L C D Q R T N D P C L G N K C V H G T - C L P I N H-Slit1

1373 N A R D G Y Q C K C K H G Q R G R Y C D Q G E G S T E P - - - - - - - - - - - - D-Slit
1386 A F - - S Y S C K C L E G H G G V L C D E E E D L F N P C Q A I K C K H G K C R H-Slit1

1401 - - - - - - - - - - - - P T V T A A S - - - - - T C R K E Q V R E Y Y T E N D - D-Slit
1424 L S G L G Q P Y C E C S S G Y T G D S C D R E I S C R G E R I R D Y Y Q K Q Q G H-Slit1

1423 - - - C R S R Q P L K Y A K C V G G C - G N Q C C A A K I V R R R K V R M V C S D-Slit
1464 Y A A C Q T T K K V S R L E C R G G C A G G Q C C G P L R S K R R K Y S F E C T H-Slit1

1459 N N R K Y I K N L D I V R K C G C T K K C Y D-Slit
1504 D G S S F V D E V E K V V K C G C T R - C V S H-Slit1
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SEQ ID NO: 1 & 2

SEQ ID NO: 1

Sequence of Human Slit-1

SEQ ID NO: 2

DNA sequence and predicted protein product. Base pair and amino acid number are indicated on the right hand side.

ATGCGCGGCGTTGGCTGGCAGATGCTGTCCCTGTCGCTGGGGTTAGTGCTGGCGATCCTGAACAAGGTGGCACCG 75
M R G V G W Q M L S L S L G L V L A I L N K V A P 25

CAGGCGTGCCCGGCGCAGTGCTCTTGCTCGGGCAGCACAGTGGACTGTCACGGGCTGGCGCTGCGCAGCGTGCCC 150
Q A C P A Q C S C S G S T V D C H G L A L R S V P 50

AGGAATATCCCCCGCAACACCGAGAGACTGGATTTAAATGGAAATAACATCACAAGAATTACGAAGACAGATTTT 225
R N I P R N T E R L D L N G N N I T R I T K T D F 75

GCTGGTCTTAGACATCTAAGAGTTCTTCAGCTTATGGAGAATAAGATTAGCACCATTGAAAGAGGAGCATTCCAG 300
A G L R H L R V L Q L M E N K I S T I E R G A F Q 100

GATCTTAAAGAACTAGAGAGACTGCGTTTAAACAGAAATCACCTTCAGCTGTTTCCTGAGTTGCTGTTTCTTGGG 375
D L K E L E R L R L N R N H L Q L F P E L L F L G 125

ACTGCGAAGCTATACAGGCTTGATCTCAGTGAAAACCAAATTCAGGCAATCCCAAGGAAAGCTTTCCGTGGGGCA 450
T A K L Y R L D L S E N Q I Q A I P R K A F R G A 150

GTTGACATAAAAAATTTGCAACTGGATTACAACCAGATCAGCTGTATTGAAGATGGGGCATTCAGGGCTCTCCGG 525
V D I K N L Q L D Y N Q I S C I E D G A F R A L R 175

GACCTGGAAGTGCTCACTCTCAACAATAACAACATTACTAGACTTTCTGTGGCAAGTTTCAACCATATGCCTAAA 600
D L E V L T L N N N N I T R L S V A S F N H M P K 200

CTTAGGACTTTTCGACTGCATTCAAACAACCTGTATTGTGACTGCCACCTGGCCTGGCTCTCCGACTGGCTTCGC 675
L R T F R L H S N N L Y C D C H L A W L S D W L R 225

AAAAGGCCTCGGGTTGGTCTGTACACTCAGTGTATGGGCCCCTCCCACCTGAGAGGCCATAATGTAGCCGAGGTT 750
K R P R V G L Y T Q C M G P S H L R G H N V A E V 250

CAAAAACGAGAATTTGTCTGCAGTGATGAGGAAGAAGGTCACCAGTCATTTATGGCTCCTTCTTGTAGTGTTTTG 825
Q K R E F V C S D E E E G H Q S F M A P S C S V L 275

CACTGCCCTGCCGCCTGTACCTGTAGCAACAATATCGTAGACTGTCGTGGGAAAGGTCTCACTGAGATCCCCACA 900
H C P A A C T C S N N I V D C R G K G L T E I P T 300

AATCTTCCAGAGACCATCACAGAAATACGTTTGGAACAGAACACAATCAAAGTCATCCCTCCTGGAGCTTTCTCA 975
N L P E T I T E I R L E Q N T I K V I P P G A F S 325

CCATATAAAAAGCTTAGACGAATTGACCTGAGCAATAATCAGATCTCTGAACTTGCACCAGATGCTTTCCAAGGA 1050
P Y K K L R R I D L S N N Q I S E L A P D A F Q G 350

CTACGCTCTCTGAATTCACTTGTCCTCTATGGAAATAAAATCACAGAACTCCCCAAAAGTTTATTTGAAGGACTG 1125
L R S L N S L V L Y G N K I T E L P K S L F E G L 375

TTTTCCTTACAGCTCCTATTATTGAATGCCAACAAGATAAACTGCCTTCGGGTAGATGCTTTTCAGGATCTCCAC 1200
F S L Q L L L L N A N K I N C L R V D A F Q D L H 400

AACTTGAACCTTCTCTCCCTATATGACAACAAGCTTCAGACCATCGCCAAGGGGACCTTTTCACCTCTTCGGGCC 1275
N L N L L S L Y D N K L Q T I A K G T F S P L R A 425

ATTCAAACTATGCATTTGGCCCAGAACCCCTTTATTTGTGACTGCCATCTCAAGTGGCTAGCGGATTATCTCCAT 1350
I Q T M H L A Q N P F I C D C H L K W L A D Y L H 450

ACCAACCCGATTGAGACCAGTGGTGCCCGTTGCACCAGCCCCCGCCGCCTGGCAAACAAAAGAATTGGACAGATC 1425
T N P I E T S G A R C T S P R R L A N K R I G Q I 475

AAAAGCAAGAAATTCCGTTGTTCAGGTACAGAAGATTATCGATCAAAATTAAGTGGAGACTGCTTTGCGGATCTG 1500
K S K K F R C S G T E D Y R S K L S G D C F A D L 500

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GCTTGCCCTGAAAAGTGTCGCTGTGAAGGAACCACAGTAGATTGCTCTAATCAAAAGCTCAACAAAATCCCGGAG  1575
 A  C  P  E  K  C  R  C  E  G  T  T  V  D  C  S  N  Q  K  L  N  K  I  P  E   525

CACATTCCCCAGTACACTGCAGAGTTGCGTCTCAATAATAATGAATTTACCGTGTTGGAAGCCACAGGAATCTTT  1650
 H  I  P  Q  Y  T  A  E  L  R  L  N  N  N  E  F  T  V  L  E  A  T  G  I  F   550

AAGAAACTTCCTCAATTACGTAAAATAAACTTTAGCAACAATAAGATCACAGATATTGAGGAGGGAGCATTTGAA  1725
 K  K  L  P  Q  L  R  K  I  N  F  S  N  N  K  I  T  D  I  E  E  G  A  F  E   575

GGAGCATCTGGTGTAAATGAAATACTTCTTACGAGTAATCGTTTGGAAAATGTGCAGCATAAGATGTTCAAGGGA  1800
 G  A  S  G  V  N  E  I  L  L  T  S  N  R  L  E  N  V  Q  H  K  M  F  K  G   600

TTGGAAAGCCTCAAAACTTTGATGTTGAGAAGCAATCGAATAACCTGTGTGGGGAATGACAGTTTCATAGGACTC  1875
 L  E  S  L  K  T  L  M  L  R  S  N  R  I  T  C  V  G  N  D  S  F  I  G  L   625

AGTTCTGTGCGTTTGCTTTCTTTGTATGATAATCAAATTACTACAGTTGCACCAGGGGCATTTGATACTCTCCAT  1950
 S  S  V  R  L  L  S  L  Y  D  N  Q  I  T  T  V  A  P  G  A  F  D  T  L  H   650

TCTTTATCTACTCTAAACCTCTTGGCCAATCCTTTTAACTGTAACTGCTACCTGGCTTGGTTGGGAGAGTGGCTG  2025
 S  L  S  T  L  N  L  L  A  N  P  F  N  C  N  C  Y  L  A  W  L  G  E  W  L   675

AGAAAGAAGAGAATTGTCACGGGAAATCCTAGATGTCAAAAACCATACTTCCTGAAAGAAATACCCATCCAGGAT  2100
 R  K  K  R  I  V  T  G  N  P  R  C  Q  K  P  Y  F  L  K  E  I  P  I  Q  D   700

GTGGCCATTCAGGACTTCACTTGTGATGACGGAAATGATGACAATAGTTGCTCCCCACTTTCTCGCTGTCCTACT  2175
 V  A  I  Q  D  F  T  C  D  D  G  N  D  D  N  S  C  S  P  L  S  R  C  P  T   725

GAATGTACTTGCTTGGATACAGTCGTCCGATGTAGCAACAAGGGTTTGAAGGTCTTGCCGAAAGGTATTCCAAGA  2250
 E  C  T  C  L  D  T  V  V  R  C  S  N  K  G  L  K  V  L  P  K  G  I  P  R   750

GATGTCACAGAGTTGTATCTGGATGGAAACCAATTTACACTGGTTCCCAAGGAACTCTCCAACTACAAACATTTA  2325
 D  V  T  E  L  Y  L  D  G  N  Q  F  T  L  V  P  K  E  L  S  N  Y  K  H  L   775

ACACTTATAGACTTAAGTAACAACAGAATAAGCACGCTTTCTAATCAGAGCTTCAGCAACATGACCCAGCTCCTC  2400
 T  L  I  D  L  S  N  N  R  I  S  T  L  S  N  Q  S  F  S  N  M  T  Q  L  L   800

ACCTTAATTCTTAGTTACAACCGTCTGAGATGTATTCCTCCTCGCACCTTTGATGGATTAAAGTCTCTTCGATTA  2475
 T  L  I  L  S  Y  N  R  L  R  C  I  P  P  R  T  F  D  G  L  K  S  L  R  L   825

CTTTCTCTACATGGAAATGACATTTCTGTTGTGCCTGAAGGTGCTTTCAATGATCTTTCTGCATTATCACATCTA  2550
 L  S  L  H  G  N  D  I  S  V  V  P  E  G  A  F  N  D  L  S  A  L  S  H  L   850

GCAATTGGAGCCAACCCTCTTTACTGTGATTGTAACATGCAGTGGTTATCCGACTGGGTGAAGTCGGAATATAAG  2625
 A  I  G  A  N  P  L  Y  C  D  C  N  M  Q  W  L  S  D  W  V  K  S  E  Y  K   875

GAGCCTGGAATTGCTCGTTGTGCTGGTCCTGGAGAAATGGCAGATAAACTTTTACTCACAACTCCCTCCAAAAAA  2700
 E  P  G  I  A  R  C  A  G  P  G  E  M  A  D  K  L  L  L  T  T  P  S  K  K   900

TTTACCTGTCAAGGTCCTGTGGATGTCAATATTCTAGCTAAGTGTAACCCCTGCCTATCAAATCCGTGTAAAAAT  2775
 F  T  C  Q  G  P  V  D  V  N  I  L  A  K  C  N  P  C  L  S  N  P  C  K  N   925

GATGGCACATGTAATAGTGATCCAGTTGACTTTTACCGATGCACCTGTCCATATGGTTTCAAGGGGCAGGACTGT  2850
 D  G  T  C  N  S  D  P  V  D  F  Y  R  C  T  C  P  Y  G  F  K  G  Q  D  C   950

GATGTCCCAATTCATGCCTGCATCAGTAACCCATGTAAACATGGAGGAACTTGCCACTTAAAGGAAGGAGAAGAA  2925
 D  V  P  I  H  A  C  I  S  N  P  C  K  H  G  G  T  C  H  L  K  E  G  E  E   975

GATGGATTCTGGTGTATTTGTGCTGATGGATTTGAAGGAGAAAATTGTGAAGTCAACGTTGATGATTGTGAAGAT  3000
 D  G  F  W  C  I  C  A  D  G  F  E  G  E  N  C  E  V  N  V  D  D  C  E  D  1000

AATGACTGTGAAAATAATTCTACATGTGTCGATGGCATTAATAACTACACATGCCTTTGCCCACCTGAGTATACA  3075
 N  D  C  E  N  N  S  T  C  V  D  G  I  N  N  Y  T  C  L  C  P  P  E  Y  T  1025
```

GGTGAGTTGTGTGAGGAGAAGCTGGACTTCTGTGCCCAGGACCTGAACCCCTGCCAGCACGATTCAAAGTGCATC 3150
G E L C E E K L D F C A Q D L N P C Q H D S K C I 1050

CTAACTCCAAAGGGATTCAAATGTGACTGCACACCAGGGTACGTAGGTGAACACTGCGACATCGATTTTGACGAC 3225
L T P K G F K C D C T P G Y V G E H C D I D F D D 1075

TGCCAAGACAACAAGTGTAAAAACGGAGCCCACTGCACAGATGCAGTGAACGGCTATACGTGCATATGCCCCGAA 3300
C Q D N K C K N G A H C T D A V N G Y T C I C P E 1100

GGTTACAGTGGCTTGTTCTGTGAGTTTTCTCCACCCATGGTCCTCCCTCGTACCAGCCCCTGTGATAATTTTGAT 3375
G Y S G L F C E F S P P M V L P R T S P C D N F D 1125

TGTCAGAATGGAGCTCAGTGTATCGTCAGAATAAATGAGCCAATATGTCAGTGTTTGCCTGGCTATCAGGGAGAA 3450
C Q N G A Q C I V R I N E P I C Q C L P G Y Q G E 1150

AAGTGTGAAAAATTGGTTAGTGTGAATTTTATAAACAAAGAGTCTTATCTTCAGATTCCTTCAGCCAAGGTTCGG 2525
K C E K L V S V N F I N K E S Y L Q I P S A K V R 1175

CCTCAGACGAACATAACACTTCAGATTGCCACAGATGAAGACAGCGGAATCCTCCTGTATAAGGGTGACAAAGAC 3600
P Q T N I T L Q I A T D E D S G I L L Y K G D K D 1200

CATATCGCGGTAGAACTCTATCGGGGGCGTGTTCGTGCCAGCTATGACACCGGCTCTCATCCAGCTTCTGCCATT 3675
H I A V E L Y R G R V R A S Y D T G S H P A S A I 1225

TACAGTGTGGAGACAATCAATGATGGAAACTTCCACATTGTGGAACTACTTGCCTTGGATCAGAGTCTCTCTTTG 3750
Y S V E T I N D G N F H I V E L L A L D Q S L S L 1250

TCCGTGGATGGTGGGAACCCCAAAATCATCACTAACTTGTCAAAGCAGTCCACTCTGAATTTTGACTCTCCACTC 3825
S V D G G N P K I I T N L S K Q S T L N F D S P L 1275

TATGTAGGAGGCATGCCAGGGAAGAGTAACGTGGCATCTCTGCGCCAGGCCCCTGGGCAGAACGGAACCAGCTTC 3900
Y V G G M P G K S N V A S L R Q A P G Q N G T S .F 1300

CACGGCTGCATCCGGAACCTTTACATCAACAGTGAGCTGCAGGACTTCCAGAAGGTGCCGATGCAAACAGGCATT 3975
H G C I R N L Y I N S E L Q D F Q K V P M Q T G I 1325

TTGCCTGGCTGTGAGCCATGCCACAAGAAGGTGTGTGCCCATGGCACATGCCAGCCCAGCAGCCAGGCAGGCTTC 4050
L P G C E P C H K K V C A H G T C Q P S S Q A G F 1350

ACCTGCGAGTGCCAGGAAGGATGGATGGGGCCCCTCTGTGACCAACGGACCAATGACCCTTGCCTTGGAAATAAA 4125
T C E C Q E G W M G P L C D Q R T N D P C L G N K 1375

TGCGTACATGGCACCTGCTTGCCCATCAATGCGTTCTCCTACAGCTGTAAGTGCTTGGAGGGCCATGGAGGTGTC 4200
C V H G T C L P I N A F S Y S C K C L E G H G G V 1400

CTCTGTGATGAAGAGGAGGATCTGTTTAACCCATGCCAGGCGATCAAGTGCAAGCATGGGAAGTGCAGGCTTTCA 4275
L C D E E E D L F N P C Q A I K C K H G K C R L S 1425

GGTCTGGGGCAGCCCTACTGTGAATGCAGCAGTGGATACACGGGGGACAGCTGTGATCGAGAAATCTCTTGTCGA 4350
G L G Q P Y C E C S S G Y T G D S C D R E I S C R 1450

GGGGAAAGGATAAGAGATTATTACCAAAAGCAGCAGGGCTATGCTGCTTGCCAAACAACCAAGAAGGTGTCCCGA 4425
G E R I R D Y Y Q K Q Q G Y A A C Q T T K K V S R 1475

TTAGAGTGCAGAGGTGGGTGTGCAGGAGGGCAGTGCTGTGGACCGCTGAGGAGCAAGCGGCGGAAATACTCTTTC 4500
L E C R G G C A G G Q C C G P L R S K R R K Y S F 1500

GAATGCACTGACGGCTCCTCCTTTGTGGACGAGGTTGAGAAAGTGGTGAAGTGCGGCTGTACGAGGTGTGTGTCC 4575
E C T D G S S F V D E V E K V V K C G C T R C V S 1525

Features of Human Slit-1 predicted protein
Co-ordinates refer to amino acid number.

Signal sequence:	7-24	
First amino-flanking sequence:	28-59	
First set of Leucine Rich Repeats:	60-179	(6 repeats)
First carboxy-flanking sequence:	180-276	
Second amino-flanking sequence:	277-308	
Second set of Leucine Rich Repeats:	309-434	(5 repeats)
Second carboxy-flanking sequence:	435-501	
Third amino-flanking sequence:	502-533	
Third set of Leucine Rich Repeats:	534-660	(5 repeats)
Third carboxy-flanking sequence:	661-722	
Fourth amino-flanking sequence:	723-754	
Fourth set of Leucine Rich Repeats:	755-855	(4 repeats)
Fourth carboxy-flanking sequence:	856-917	
First EGF repeat:	918-952	
Second EGF repeat:	953-993	
Third EGF repeat:	994-1031	
Fourth EGF repeat:	1032-1071	
Fifth EGF repeat:	1072-1109	
Spacer:	1110-1116	
Sixth EGF repeat:	1117-1154	
"99aa spacer":	1155-1329	
Seventh EGF repeat:	1330-1366	
Eighth EGF repeat:	1367-1404	
Nineth EGF repeat:	1405-1447	
Cysteine knot motif:	1448-1525	

Leucine rich repeats (LRRs) are predicted by comparison with known proteins and by the presence of the core sequence: xxxFxxLxxLxxLxLxxNxIxxL, where x is any amino acid. In slit proteins, the LRRs are flanked by conserved sequences referred to as the amino- and carboxy- flanking regions. These flanking regions are found in other known proteins, but only in a few instances are both the amino- and carboxy- flank regions present in a single protein. The amino flank region is defined by the consensus: CPxxCxC[1-6x]GxxVDCxxxGL[2-4x]αPxxαPxdttx where x is any amino acid, [x] represents a variable number of amino acids and α is a hydrophobic residue. Lower case indicates a residue is not highly conserved at a particular position. The carboxy flank region is defined by the consensus: PßxCγCxα[1-5x]Wα[14-26x]RCxxPxxxxxxxαxxαxxxxF[1-3x]Cs[3-17x] where ß is W or a hydrophobic residue, γ is D or N and α is a hydrophobic residue.
Epidermal growth factor (EGF) repeats are predicted by the consensus: CxxxxCxngxC[6-9x]αxCxCxxGαxGxxCxxxxxx.

The so called "99aa spacer" is actually ~200 amino acids in the Drosophila protein and 174 amino acids in Human Slit-1. This region shows homology to the G-loops of laminin A chains.

Cysteine knots are dimerisation domains defined by the presence of six cysteine residues between which disulphide bridges form. The only absolutely conserved residues are the six cysteines, and spacing between them is highly variable, apart from between cysteines 2 and 3, and 5 and 6: C[x]C[1-3x]GxC[x]C[x]CxC. The glycine between cysteines 2 and 3 is only present in a subset of cysteine knots. Drosophila slit and Human slit-1 both have an extra cysteine after cysteines 5 and 6: this may serve as an intermolecular bond.

Human Slit-1 gene displays the overall structure of the Drosophila gene, and amino acid conservation is found along the entire length of the protein (48% homology at the amino acid sequence excluding the signal sequence; see below). The Human gene has an extra LRR between LRR2 and LRR3 of the first set of LRRs; in the third set, the Human gene has an extra LRR between LRR3 and LRR4. The Human gene has two extra EGF repeats, on either side of the seventh EGF repeat in Drosophila slit.

Isolation of Human slit-1

Searching of the EST database revealed an EST, ab16g10.r1, with homology to the 99aa spacer region of Drosophila slit. This EST was used to probe a Human fetal brain library (Stratagene), and clones for Human slit-1 were isolated.

Amino acid identity between Drosophila Slit and Human Slit-1

First amino-flanking sequence:	53%	
First set of Leucine Rich Repeats:	52%	(54%, 67%, NA, 38%, 54%, 50%)
First carboxy-flanking sequence:	42%	
Second amino-flanking sequence:	50%	
Second set of Leucine Rich Repeats:	60%	(54%, 58%, 67%, 71%, 50%)
Second carboxy-flanking sequence:	62%	
Third amino-flanking sequence:	56%	
Third set of Leucine Rich Repeats:	49%	(46%, 46%, 42%, NA, 58%)
Third carboxy-flanking sequence:	36%	
Fourth amino-flanking sequence:	53%	
Fourth set of Leucine Rich Repeats:	48%	(25%, 58%, 46%, 63%)
Fourth carboxy-flanking sequence:	63%	
First EGF repeat:	34%	
Second EGF repeat:	46%	
Third EGF repeat:	46%	
Fourth EGF repeat:	35%	
Fifth EGF repeat:	47%	
Spacer:	22%	
Sixth EGF repeat:	40%	
"99aa spacer":	38%	
Seventh EGF repeat:	11%/NA	
Eighth EGF repeat:	44%	
Nineth EGF repeat:	29%/NA	
Cysteine knot motif:	34%	

NA: not applicable due to absence of homologous repeat.
Figures for individual LRRs are shown in brackets.

TABLE 3

Alignment of Slit sequences

```
1    MAAPSRTTLMPPPPFRLQLRL-LILPILLLLLRHDAVHAEPY  D-Slit
1    MRGVGWQ-------MLSLSLGLVLAIL-------------  H-Slit1

40   SGGFGSSAVSSGGLGSVGIHIPGGGVGVITEARCPRVCSC  D-Slit
21   --------------------------NKVAPQACPAQCSC  H-Slit1

80   TGLNVDCSHRGLTSVPRKISADVERLELQGNNLTVIYETD  D-Slit
35   SGSTVDCHGLALRSVPRNIPRNTERLDLNGNNITRITKTD  H-Slit1

120  FQRLTKLRMLQLTDNQIHTIERNSFQDLVSLERL------  D-Slit
75   FAGLRHLRVLQLMENKISTIERGAFQDLKELERLRLNRNH  H-Slit1
1         HLRVLQLMENRISTIERGAFQDLKELERLRLNRNN  M-Slit1

154   ------------------DISNNVITTVGRRVFKGAQSLR  D-Slit
115  LQLFPELLFLGTAKLYRLDLSENQIQAIPRKAFRGAVDIK  H-Slit1
36   LQLFPELLFLGTARLYRLDLSENQIQAIPRKAFRGAVDIK  M-Slit1

176  SLQLDNNQITCLDEHAFKGLVELEILTLNNNNLTSLPHNI  D-Slit
155  NLQLDYNQISCIEDGAFRALRDLEVLTLNNNNITRLSVAS  H-Slit1
76   NLQLDYNQISCIEDGAFRALRDLEVLTLNNNNITRLSVAS  M-Slit1

216  FGGLGRLRALRLSDNPFACDCHLSWLSRFLRSATRLAPYT  D-Slit
195  FNHMPKLRTFRLHSNNLYCDCHLAWLSDWLRKRPRVGLYT  H-Slit1
116  FNHMPKLRTFRLHSNNLYC                       M-Slit1

256  RCQSPSQLKGQNVADLHDQEFKCSGLTE-HAPM---ECGA  D-Slit
235  QCMGPSHLRGHNVAEVQKREFVCSDEEEGHQSFMAPSCSV  H-Slit1

292  ENSCPHPCRCADGIVDCREKSLTSVPVTLPDDTTDVRLEQ  D-Slit
275  LH-CPAACTCSNNIVDCRGKGLTEIPTNLPETITEIRLEQ  H-Slit1
1       SPCTCSNNIVDCRGKGLMEIPANLPEGIVEIRLEQ  H-Slit2

332  NFITELPPKSFSSFRRLRRIDLSNNNISRIAHDALSGLKQ  D-Slit
314  NTIKVIPPGAFSPYKKLRRIDLSNNQISELAPDAFQGLRS  H-Slit1
36   NSIKAIPAGAFTQYKKLKRIDISKNQISDIAPDAFQGLKS  H-Slit2

372  LTTLVLYGNKIKDLPSGVFKGLGSLRLLLLNANEISCIRK  D-Slit
354  LNSLVLYGNKITELPKSLFEGLFSLQLLLLNANKINCLRV  H-Slit1
76   LTSLVLYGNKITEIAKGLFDGLVSLQLLLL            H-Slit2

1                                           R  CE-Slit
412  DAFRDLHSLSLLSLYDNNIQSLANGTFDAMKSMKTVHLAK  D-Slit-
394  DAFQDLHNLNLLSLYDNKLQTIAKGTFSPLRAIQTMHLAQ  H-Slit1

2    NPXICDCNLQWLAQINLQKNIETSGARCEQPKRLRKKKFA  CE-Slit
452  NPFICDCNLRWLADYLHKNPIETSGARCESPKRMHRRRIE  D-Slit
434  NPFICDCHLKWLADYLHTNPIETSGARCTSPRRLANKRIG  H-Slit1

42   TLPPNKFKCKGSESFVSMYADSCFIDSICPTQCDCYGTTV  CE-Slit
492  SLREEKFKCS-WGELRMKLSGECRMDSDCPAMCHCEGTTV  D-Slit
474  QIKSKKFRCSGTEDYRSKLSGDCFADLACPEKCRCEGTTV  H-Slit1
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82   D C N K R G L N T I P T S I P R F A T Q L L L S G N N I S T V D L N S N I H V L   CE-Slit
531  D C T G R R L K E I P R D I P L H T T E L L L N D N E L G R I S S D G L F G R L   D-Slit
514  D C S N Q K L N K I P E H I P Q Y T A E L R L N N N E F T V L E A T G I F K K L   H-Slit1

122  E N L E X L D L S N N H I T F I N D K S F E K L S K L R E L X L N D               CE-Slit
571  P H L V K L E L K R N Q L T G I E P N A F E G A S H I Q E L Q L G E N K I K E I   D-Slit
554  P Q L R K I N F S N N K I T D I E E G A F E G A S G V N E I L L T S N R L E N V   H-Slit1
1                                      E G A F N G A A S V Q E L M L T G N Q L E T V   H-Slit2

611  S N K M F - - - - - - - - - - - - - - - - - - - - - - - - - L G L H Q L K T L N   D-Slit
594  Q H K M F K G - L E S L K T L M L R S N R I T C V G N D S F I G L S S V R L L S   H-Slit1
24   H G R G F R G G L S G L K T L M L R S N L I G C V S N D T F A G L S S V R L L S   H-Slit2

626  L Y D N Q I S C V M P G S F E H L N S L T S L N L A S N P F N C N C H L A W - F   D-Slit
633  L Y D N Q I T T V A P G A F D T L H S L S T L N L L A N P F N C N C Y L A W - L   H-Slit1
64   L Y D N R I T T I T P G A F T T L V S L S T I N L L S N P F N C N C H L G A G L   H-Slit2

665  A E C V R K K S L N G G A A R C G A P S K V R D V Q I K D L P H S E F K C S S E   D-Slit
672  G E W L R K K R I V T G N P R C Q K P Y F L K E I P I Q D V A I Q D F T C D D G   H-Slit1
104  G K W L R K R R I V S G N P R C Q K P F F L K E I P I Q G V G H P G I             H-Slit2

1                                                      S N K N L T S F P S R I P F D   CE-Slit
705  N S E - G C L G D G Y C P P S C T C T G T V V A C S R N Q L K E I P R G I P A E   D-Slit
712  N D D N S C S P L S R C P T E C T C L D T V V R C S N K G L K V L P K G I P R D   H-Slit1

16   T T E L Y L D A N Y I N E I P A H D L N R L Y S L T K L D L S H N R L I S L E N   CE-Slit
744  T S E L Y L E S N E I E Q I H Y E R I R H L R S L T R L D L S N N Q I T I L S N   D-Slit
752  V T E L Y L D G N Q F T L V P K E - L S N Y K H L T L I D L S N N R I S T L S N   H-Slit1

56   N T F S N L T R L S T L I I S Y N K L R C L Q P L A F N G L N A L R I L S L H G   CE-Slit
784  Y T F A N L T K L S T L I I S Y N K L Q C L Q R H A L S G L N N L R V V S L H G   D-Slit
791  Q S F S N M T Q L L T L I L S Y N R L R C I P P R T F D G L K S L R L L S L H G   H-Slit1

96   N D I S F L P Q S A F S N L T S I T H I A V G S N S L Y C D C N M A W F S K W I   CE-Slit
824  N R I S M L P E G S F E D L K S L T H I A L G S N P L Y C D C G L K W F S D W I   D-Slit
831  N D I S V V P E G A F N D L S A L S H L A I G A N P L Y C D C N M Q W L S D W V   H-Slit1

136  K S K F I E A G I A R C E Y P N T V S N Q L L L T A Q P Y Q F T C D S K V P T K   CE-Slit
864  K L D Y V E P G I A R C A E P E Q M K D K L I L S T P S S S F V C R G R V R N D   D-Slit
871  K S E Y K E P G I A R C A G P G E M A D K L L L T T P S K K F T C Q G P V D V N   H-Slit1

176  L A T K C D L C L N S P C K N N A I C E T T S S R K Y T C N C T P G F Y G V H C   CE-Slit
904  I L A K C N A C F E Q P C Q N Q A Q C V A L P Q R E Y Q C L C Q P G Y H G K H C   D-Slit
911  I L A K C N P C L S N P C K N D G T C N S D P V D F Y R C T C P Y G F K G Q D C   H-Slit1

216  E N Q I D A C Y G S P C L N N A T C K V - - A Q A G R F N C Y C N K G F E G D Y   CE-Slit
944  E F M I D A C Y G N P C R N N A T C T V L E - - E G R F S C Q C A P G Y T G A R   D-Slit
951  D V P I H A C I S N P C K H G G T C H L K E G E E D G F W C I C A D G F E G E N   H-Slit1

254  C E K N I D D C V - N S K C E N G G K C V D L V R F C S E E L K N F Q S F Q I N   CE-Slit
982  C E T N I D D C L G E I K C Q N N A T C I D - - - - - - - - - - - - - - - G V E   D-Slit
991  C E V N V D D C - E D N D C E N N S T C V D - - - - - - - - - - - - - - - G I N   H-Slit1
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293   S Y R C D C P M E Y E G K H C E D K L E Y C T K K L N P C E N N G K C I P I N G   CE-Slit
1007  S Y K C E C Q P G F S G E F C D T K I Q F C S P E F N P C A N G A K C M D H F T   D-Slit
1015  N Y T C L C P P E Y T G E L C E E K L D F C A Q D L N P C Q H D S K C I L T P K   H-Slit1
1                                                                          D P L P V   M-Slit2

333   S Y S C M C S P G F T G N N C E T N I D D C K N V E C Q N G G S C V D G I L S Y   CE-Slit
1047  H Y S C D C Q A G F H G T N C T D N I D D C Q N H M C Q N G G T C V D G I N D Y   D-Slit
1055  G F K C D C T P G Y V G E H C D I D F D D C Q D N K C K N G A H C T D A V N G Y   H-Slit1
1                                   N N D D C V G H K C R H G A Q C V D E V N G Y   M-Slit1
1     W P R C E C M P G Y A G D N C S E N Q D D C R D H R C Q N G A Q C M D E V N S Y   H-Slit2
6     H H R C E C M L G Y T G D N C S E N Q D D C K D H K C Q N G A Q C V D E V N S Y   M-Slit2

373   D C L C R P G Y A G Q Y C E I P P M M D M E Y Q K T D A C Q Q S A C G Q G - E C   CE-Slit
1087  Q C R C P D D Y T G K Y C E G H N M I S M M Y P Q T S P C Q N H E C K H G V - C   D-Slit
1095  T C I C P E G Y S G L F C E F S P - - P M V L P R T S P C D N F D C Q N G A Q C   H-Slit1
24    T C I C P Q G F S G L F C E H P P - - P M V L L Q T S P C D Q Y E C Q N G A Q C   M-Slit1
41    S C L C A E G Y S G Q L C E I P P - - H L P A P K - S P C E G T E C Q N G A N C   H-Slit2
46    A C L C V E G Y S G Q L C E I P P - - - - - A P R - S S C E G T E C Q N G A N C   M-Slit2

412   V A S Q N - S S D F T C K C H E G F S G P S C D R Q M S V G F K N P G A Y L A L   CE-Slit
1126  F Q P N A Q G S D Y L C R C H P G Y T G K W C E Y L T S I S F V H N N S F V E L   D-Slit
1133  I V R I N E P - - - I C Q C L P G Y Q G E K C E K L V S V N F I N K E S Y L Q I   H-Slit1
62    I V V Q Q E P - - - T C R C P P G F A G P R C E K L I T V N F V G K D S Y V E L   M-Slit1
78    V D Q G N R P - - - V C Q C L P G F G G P E C E K L L S V N F V D R D T Y L Q F   H-Slit2
80    V D Q G S R P - - - V C Q C L P G F G G P E C E K L L S V N F V D R D T Y L Q F   M-Slit2

451   D P L A S - - D G T I T M T L R T T S K I G I L L Y Y G D D H F V S A E L Y D G   CE-Slit
1166  E P L R T R P E A N V T I V F S S A E Q N G I L M Y D G Q D A H L A V E L F N G   D-Slit
1170  P S A K V R P Q T N I T L Q I A T D E D S G I L L Y K G D K D H I A V E L Y R G   H-Slit1
99    A S A K V R                                                                       M-Slit1
115   T D L Q N W X R X N I T L Q V F T A E D N G I L L Y N G G N D H I A V X L Y X G   H-Slit2
117   T D L Q N W P R A N I T L Q V S T A E D N G I L L Y N G D N D H I A V E L Y       M-Slit2

489   R V K L V Y Y I G N F P A S H M Y S S V K V N D G L P H R I S I R T S E R K C F   CE-Slit
1206  R I R V S Y D V G N H P V S T M Y S F E M V A D G K Y H A V E L L A I K K N F T   D-Slit
1210  R V R A S Y D T G S H P A S A I Y S V E T I N D G N F H I V E L L A L D Q S L S   H-Slit1
155   H V R F S Y                                                                       H-Slit2

529   L Q I D K N P V Q I V E N S G K S D Q L I T K G K E M L Y I G G L P I E K S Q D   CE-Slit
1246  L R V D R G L A R S I I N E G S N D Y L - - K L T T P M F L G G L P V D P A Q Q   D-Slit
1250  L S V D G G N P K I I T N L S K Q S T L - - N F D S P L Y V G G M P G K S N V A   H-Slit1
1                                                                      I L D V A   M-Slit1

569   A K R R F H V K N S E S L K G C I S S I T I N E V P I N L Q Q A L E N V N T E Q   CE-Slit
1284  A Y K N W Q I R N L T S F K G C M K E V W I N H K L V D F G N A Q R Q Q K I T P   D-Slit
1288  S L R Q A P G Q N G T S F H G C I R N L Y I N S E L Q D F Q K V P M Q T G I L P   H-Slit1
6     S L R Q A P G E N G T S F H G C I R N L Y I N S E L Q D F R K M P M Q T G I L P   M-Slit1

609   S C - - - - - - - - - - - - - - - S A T V N F - - - - - - - - - - - - - - - - -   CE-Slit
1324  G C A L - - - - L E G E Q Q E E E D D E Q D F M D E - - - - - - - T P H I K E E P   D-Slit
1328  G C E P C H K K V C A H G T C Q P S S Q A G F T C E C Q E G W M G P L C D Q R T   H-Slit1
46    G C E P C H K K V C A H G C C Q P S S Q S G F T C E C E E G W M G P L C D Q R T   M-Slit1
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617   - - - C A C I D C G N G - K C T N N A L S P K G Y M C Q C D S H F S G E H C D E   CE-Slit
1354  V D P C L E N K C R R G S R C V P N S N A R D G Y Q C K C K H G Q R G R Y C D Q   D-Slit
1368  N D P C L G N K C V H G T - C L P I N A F - - S Y S C K C L E G H G G V L C D E   H-Slit1
86    N D P C L G N K C V H G T - C L P I N A F - - S Y S C K C L E G H G G V L C D E   M-Slit1

653   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   CE-Slit
1394  G E G S T E P - - - - - - - - - - - - - - - - - - - - - - - - - P T V T A A S - -   D-Slit
1405  E E D L F N P C Q A I K C K H G K C R L S G L G Q P Y C E C S S G Y T G D S C D   H-Slit1
123   E E D L F N P C Q M I K C K H G K C R L S G V G Q P Y C E C N S G F T G D S C D   M-Slit1
1                                     Q C H I S D Q G E P Y C L C Q P G F S G E H C Q   H-Slit2
1                   A F K C H H G Q C H I S D R G E P Y C L C Q P G F S G H H C E   M-Slit2

653   K R I K C D K Q K F R R H H I E N E - - - - C R S V D R I K I A E C N G Y C G G   CE-Slit
1408  T - - - C R K E Q V R E Y Y T E N D - - - - C R S R Q P L K Y A K C V G G C G -   D-Slit
1445  R E I S C R G E R I R D Y Y Q K Q Q G Y A A C Q T T K K V S R L E C R G G C A G   H-Slit1
163   R E I S C R G E R I R D Y Y Q K Q Q G Y A A C Q T T K K V S R L E C R G G C A G   M-Slit1
25    Q E N P C L G Q V V R E V I R R Q K G Y A S C A T A S K V P I M E C R G G C - G   H-Slit2
32    Q E N P C M G E I V R E A I R R Q K D Y A S C A T A S K V P I M E C R G G C - G   M-Slit2

689   E Q N C C T A V K K K Q R K V K M I C K N G T T K I S T V H I I R Q C Q C E P T   CE-Slit
1440  - N Q C C A A K I V R R R K V R M V C S N N R K Y I K N L D I V R K C G C - - T   D-Slit
1485  G Q - C C G P L R S K R R K Y S F E C T D G S S F V D E V E K V V K C G C T R -   H-Slit1
203   G Q - C C G P L R S K R R K Y S F E C T D G S S F V D E V E K V V K C G C A R -   M-Slit1
64    P Q - C C Q P T R S K R R K Y V F Q C T D G S S F V E E V E R H L E C G C L A -   H-Slit2
71    T T - C C Q P I R S K R R K Y V F Q C T D G S S F V E E V E R H L E C G C R A -   M-Slit2

729   K S V L S E K                                                                     CE-Slit
1477  K K C Y                                                                           D-Slit
1523  - - C V S                                                                         H-Slit1
241   - - C A S                                                                         M-Slit1
102   - - C - S                                                                         H-Slit2
109   - - C - S                                                                         M-Slit2
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TABLE 4

Alignment of Drosophila Slit and Human Slit-1

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1    M A A P S R T T L M P P P F R L Q L R L - L I L P I L L L L R H D A V H A E P Y   D-Slit
1    M R G V G W Q - - - - - - - M L S L S L G L V L A I L - - - - - - - - - - - - -   H-Slit1

40   S G G F G S S A V S S G G L G S V G I H I P G G G V G V I T E A R C P R V C S C   D-Slit
21   - - - - - - - - - - - - - - - - - - - - - - - - - - N K V A P Q A C P A Q C S C   H-Slit1

80   T G L N V D C S H R G L T S V P R K I S A D V E R L E L Q G N N L T V I Y E T D   D-Slit
35   S G S T V D C H G L A L R S V P R N I P R N T E R L D L N G N N I T R I T K T D   H-Slit1

120  F Q R L T K L R M L Q L T D N Q I H T I E R N S F Q D L V S L E R L - - - - - -   D-Slit
75   F A G L R H L R V L Q L M E N K I S T I E R G A F Q D L K E L E R L R L N R N H   H-Slit1

154  - - - - - - - - - - - - - - - - - - D I S N N V I T T V G R R V F K G A Q S L R   D-Slit
115  L Q L F P E L L F L G T A K L Y R L D L S E N Q I Q A I P R K A F R G A V D I K   H-Slit1

176  S L Q L D N N Q I T C L D E H A F K G L V E L E I L T L N N N N L T S L P H N I   D-Slit
155  N L Q L D Y N Q I S C I E D G A F R A L R D L E V L T L N N N N I T R L S V A S   H-Slit1

216  F G G L G R L R A L R L S D N P F A C D C H L S W L S R F L R S A T R L A P Y T   D-Slit
195  F N H M P K L R T F R L H S N N L Y C D C H L A W L S D W L R K R P R V G L Y T   H-Slit1

256  R C Q S P S Q L K G Q N V A D L H D Q E F K C S G L T E - H A P M - - - E C G A   D-Slit
235  Q C M G P S H L R G H N V A E V Q K R E F V C S D E E E G H Q S F M A P S C S V   H-Slit1

292  E N S C P H P C R C A D G I V D C R E K S L T S V P V T L P D D T T D V R L E Q   D-Slit
275  L H - C P A A C T C S N N I V D C R G K G L T E I P T N L P E T I T E I R L E Q   H-Slit1

332  N F I T E L P P K S F S S F R R L R R I D L S N N N I S R I A H D A L S G L K Q   D-Slit
314  N T I K V I P P G A F S P Y K K L R R I D L S N N Q I S E L A P D A F Q G L R S   H-Slit1

372  L T T L V L Y G N K I K D L P S G V F K G L G S L R L L L L N A N E I S C I R K   D-Slit
354  L N S L V L Y G N K I T E L P K S L F E G L F S L Q L L L L N A N K I N C L R V   H-Slit1

412  D A F R D L H S L S L L S L Y D N N I Q S L A N G T F D A M K S M K T V H L A K   D-Slit
394  D A F Q D L H N L N L L S L Y D N K L Q T I A K G T F S P L R A I Q T M H L A Q   H-Slit1

452  N P F I C D C N L R W L A D Y L H K N P I E T S G A R C E S P K R M H R R R I E   D-Slit
434  N P F I C D C H L K W L A D Y L H T N P I E T S G A R C T S P R R L A N K R I G   H-Slit1

492  S L R E E K F K C S - W G E L R M K L S G E C R M D S D C P A M C H C E G T T V   D-Slit
474  Q I K S K K F R C S G T E D Y R S K L S G D C F A D L A C P E K C R C E G T T V   H-Slit1

531  D C T G R R L K E I P R D I P L H T T E L L L N D N E L G R I S S D G L F G R L   D-Slit
514  D C S N Q K L N K I P E H I P Q Y T A E L R L N N N E F T V L E A T G I F K K L   H-Slit1

571  P H L V K L E L K R N Q L T G I E P N A F E G A S H I Q E L Q L G E N K I K E I   D-Slit
554  P Q L R K I N F S N N K I T D I E E G A F E G A S G V N E I L L T S N R L E N V   H-Slit1

611  S N K M F L G L H Q L K T L - - - - - - - - - - - - - - - - - - - - - - - - N L   D-Slit
594  Q H K M F K G L E S L K T L M L R S N R I T C V G N D S F I G L S S V R L L S L   H-Slit1

627  Y D N Q I S C V M P G S F E H L N S L T S L N L A S N P F N C N C H L A W F A E   D-Slit
634  Y D N Q I T T V A P G A F D T L H S L S T L N L L A N P F N C N C Y L A W L G E   H-Slit1
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667  C V R K K S L N G G A A R C G A P S K V R D V Q I K D L P H S E F K C S S E N S  D-Slit
674  W L R K K R I V T G N P R C Q K P Y F L K E I P I Q D V A I Q D F T C D D G N D  H-Slit1

707  E - G C L G D G Y C P P S C T C T G T V V A C S R N Q L K E I P R G I P A E T S  D-Slit
714  D N S C S P L S R C P T E C T C L D T V V R C S N K G L K V L P K G I P R D V T  H-Slit1

746  E L Y L E S N E I E Q I H Y E R I R H L R S L T R L D L S N N Q I T I L S N Y T  D-Slit
754  E L Y L D G N Q F T L V P K E - L S N Y K H L T L I D L S N N R I S T L S N Q S  H-Slit1

786  F A N L T K L S T L I I S Y N K L Q C L Q R H A L S G L N N L R V V S L H G N R  D-Slit
793  F S N M T Q L L T L I L S Y N R L R C I P P R T F D G L K S L R L L S L H G N D  H-Slit1

826  I S M L P E G S F E D L K S L T H I A L G S N P L Y C D C G L K W F S D W I K L  D-Slit
833  I S V V P E G A F N D L S A L S H L A I G A N P L Y C D C N M Q W L S D W V K S  H-Slit1

866  D Y V E P G I A R C A E P E Q M K D K L I L S T P S S S F V C R G R V R N D I L  D-Slit
873  E Y K E P G I A R C A G P G E M A D K L L L T T P S K K F T C Q G P V D V N I L  H-Slit1

906  A K C N A C F E Q P C Q N Q A Q C V A L P Q R E Y Q C L C Q P G Y H G K H C E F  D-Slit
913  A K C N P C L S N P C K N D G T C N S D P V D F Y R C T C P Y G F K G Q D C D V  H-Slit1

946  M I D A C Y G N P C R N N A T C T V L E - - E G R F S C Q C A P G Y T G A R C E  D-Slit
953  P I H A C I S N P C K H G G T C H L K E G E E D G F W C I C A D G F E G E N C E  H-Slit1

984  T N I D D C L G E I K C Q N N A T C I D G V E S Y K C E C Q P G F S G E F C D T  D-Slit
993  V N V D D C - E D N D C E N N S T C V D G I N N Y T C L C P P E Y T G E L C E E  H-Slit1

1024 K I Q F C S P E F N P C A N G A K C M D H F T H Y S C D C Q A G F H G T N C T D  D-Slit
1032 K L D F C A Q D L N P C Q H D S K C I L T P K G F K C D C T P G Y V G E H C D I  H-Slit1

1064 N I D D C Q N H M C Q N G G T C V D G I N D Y Q C R C P D D Y T G K Y C E G H N  D-Slit
1072 D F D D C Q D N K C K N G A H C T D A V N G Y T C I C P E G Y S G L F C E F S P  H-Slit1

1104 M I S M M Y P Q T S P C Q N H E C K H G V - C F Q P N A Q G S D Y L C R C H P G  D-Slit
1112 - - P M V L P R T S P C D N F D C Q N G A Q C I - - - V R I N E P I C Q C L P G  H-Slit1

1143 Y T G K W C E Y L T S I S F V H N N S F V E L E P L R T R P E A N V T I V F S S  D-Slit
1147 Y Q G E K C E K L V S V N F I N K E S Y L Q I P S A K V R P Q T N I T L Q I A T  H-Slit1

1183 A E Q N G I L M Y D G Q D A H L A V E L F N G R I R V S Y D V G N H P V S T M Y  D-Slit
1187 D E D S G I L L Y K G D K D H I A V E L Y R G R V R A S Y D T G S H P A S A I Y  H-Slit1

1223 S F E M V A D G K Y H A V E L L A I K K N F T L R V D R G L A R S I I N E G S N  D-Slit
1227 S V E T I N D G N F H I V E L L A L D Q S L S L S V D G G N P K I I T N L S K Q  H-Slit1

1263 D Y L K L T T P M F L G G L P V D P A Q Q A Y K N W Q I R N L T S F K G C M K E  D-Slit
1267 S T L N F D S P L Y V G G M P G K S N V A S L R Q A P G Q N G T S F H G C I R N  H-Slit1

1303 V W I N H K L V D F G N A Q R Q Q K I T P G C A L - - - - L E G E Q Q E E E D D  D-Slit
1307 L Y I N S E L Q D F Q K V P M Q T G I L P G C E P C H K K V C A H G T C Q P S S  H-Slit1

1339 E Q D F M D E - - - - - - T P H I K E E P V D P C L E N K C R R G S R C V P N S  D-Slit
1347 Q A G F T C E C Q E G W M G P L C D Q R T N D P C L G N K C V H G T - C L P I N  H-Slit1
```

```
1373 N A R D G Y Q C K C K H G Q R G R Y C D Q G E G S T E P - - - - - - - - - - - - - D-Slit
1386 A F - - S Y S C K C L E G H G G V L C D E E E D L F N P C Q A I K C K H G K C R H-Slit1

1401 - - - - - - - - - - - - P T V T A A S - - - - - T C R K E Q V R E Y Y T E N D - D-Slit
1424 L S G L G Q P Y C E C S S G Y T G D S C D R E I S C R G E R I R D Y Y Q K Q Q G H-Slit1

1423 - - - C R S R Q P L K Y A K C V G G C - G N Q C C A A K I V R R R K V R M V C S D-Slit
1464 Y A A C Q T T K K V S R L E C R G G C A G G Q C C G P L R S K R R K Y S F E C T H-Slit1

1459 N N R K Y I K N L D I V R K C G C T K K C Y D-Slit
1504 D G S S F V D E V E K V V K C G C T R - C V S H-Slit1
```

TABLE 5(A)

Hybridisation Probes for regions of Human Slit-1

Hybridisation Probe for the first Leucine rich repeat region

82-828

```
TGCCCGGCGCAGTGCTCTTGCTCGGCAGCACAGTGGACTGTCACGGGCTGGCGCTGCGCAGCGTGCCCAGGAAT   75
ATCCCCCGCAACACCGAGAGACTGGATTTAAATGGAAATAACATCACAAGAATTACGAAGACAGATTTTGCTGGT  150
CTTAGACATCTAAGAGTTCTTCAGCTTATGGAGAATAAGATTAGCACCATTGAAAGAGGAGCATTCCAGGATCTT  225
AAAGAACTAGAGAGACTGCGTTTAAACAGAAATCACCTTCAGCTGTTTCCTGAGTTGCTGTTTCTTGGGACTGCG  300
AAGCTATACAGGCTTGATCTCAGTGAAAACCAAATTCAGGCAATCCCAAGGAAAGCTTTCCGTGGGGCAGTTGAC  375
ATAAAAAATTTGCAACTGGATTACAACCAGATCAGCTGTATTGAAGATGGGGCATTCAGGGCTCTCCGGGACCTG  450
GAAGTGCTCACTCTCAACAATAACAACATTACTAGACTTTCTGTGGCAAGTTTCAACCATATGCCTAAACTTAGG  525
ACTTTTCGACTGCATTCAAACAACCTGTATTGTGACTGCCACCTGGCCTGGCTCTCCGACTGGCTTCGCAAAAGG  600
CCTCGGGTTGGTCTGTACACTCAGTGTATGGCCCCTCCCACCTGAGAGGCCATAATGTAGCCGAGGTTCAAAAA  675
CGAGAATTTGTCTGCAGTGATGAGGAAGAAGGTCACCAGTCATTTATGGCTCCTTCTTGTAGTGTTTTGCAC     747
```

Hybridisation Probe for the second Leucine rich repeat region

829-1503

```
TGCCCTGCCGCCTGTACCTGTAGCAACAATATCGTAGACTGTCGTGGGAAAGGTCTCACTGAGATCCCCACAAAT   75
CTTCCAGAGACCATCACAGAAATACGTTTGGAACAGAACACAATCAAAGTCATCCCTCCTGGAGCTTTCTCACCA  150
TATAAAAAGCTTAGACGAATTGACCTGAGCAATAATCAGATCTCTGAACTTGCACCAGATGCTTTCCAAGGACTA  225
CGCTCTCTGAATTCACTTGTCCTCTATGGAAATAAAATCACAGAACTCCCCAAAAGTTTATTTGAAGGACTGTTT  300
TCCTTACAGCTCCTATTATTGAATGCCAACAAGATAAACTGCCTTCGGGTAGATGCTTTTCAGGATCTCCACAAC  375
TTGAACCTTCTCTCCCTATATGACAACAAGCTTCAGACCATCGCCAAGGGGACCTTTTCACCTCTTCGGGCCATT  450
CAAACTATGCATTTGGCCCAGAACCCCTTTATTTGTGACTGCCATCTCAAGTGGCTAGCGGATTATCTCCATACC  525
AACCCGATTGAGACCAGTGGTGCCCGTTGCACCAGCCCCCGCCGCCTGGCAAACAAAAGAATTGGACAGATCAAA  600
AGCAAGAAATTCCGTTGTTCAGGTACAGAAGATTATCGATCAAAATTAAGTGGAGACTGCTTTGCGGATCTGGCT  675
```

Hybridisation Probe for the third Leucine rich repeat region

1504-2166

```
TGCCCTGAAAAGTGTCGCTGTGAAGGAACCACAGTAGATTGCTCTAATCAAAAGCTCAACAAAATCCCGGAGCAC   75
ATTCCCCAGTACACTGCAGAGTTGCGTCTCAATAATAATGAATTTACCGTGTTGGAAGCCACAGGAATCTTTAAG  150
AAACTTCCTCAATTACGTAAAATAAACTTTAGCAACAATAAGATCACAGATATTGAGGAGGGAGCATTTGAAGGA  225
GCATCTGGTGTAAATGAAATACTTCTTACGAGTAATCGTTTGGAAAATGTGCAGCATAAGATGTTCAAGGGATTG  300
GAAAGCCTCAAAACTTTGATGTTGAGAAGCAATCGAATAACCTGTGTGGGGAATGACAGTTTCATAGGACTCAGT  375
TCTGTGCGTTTGCTTTCTTTGTATGATAATCAAATTACTACAGTTGCACCAGGGGCATTTGATACTCTCCATTCT  450
TTATCTACTCTAAACCTCTTGGCCAATCCTTTTAACTGTAACTGCTACCTGGCTTGGTTGGGAGAGTGGCTGAGA  525
AAGAAGAGAATTGTCACGGGAAATCCTAGATGTCAAAAACCATACTTCCTGAAAGAAATACCCATCCAGGATGTG  600
GCCATTCAGGACTTCACTTGTGATGACGGAAATGATGACAATAGTTGCTCCCCACTTTCTCGC              663
```

Hybridisation Probe for the fourth Leucine rich repeat region

2167-2751

```
TGTCCTACTGAATGTACTTGCTTGGATACAGTCGTCCGATGTAGCAACAAGGGTTTGAAGGTCTTGCCGAAAGGT   75
ATTCCAAGAGATGTCACAGAGTTGTATCTGGATGGAAACCAATTTACACTGGTTCCCAAGGAACTCTCCAACTAC  150
AAACATTTAACACTTATAGACTTAAGTAACAACAGAATAAGCACGCTTTCTAATCAGAGCTTCAGCAACATGACC  225
CAGCTCCTCACCTTAATTCTTAGTTACAACCGTCTGAGATGTATTCCTCCTCGCACCTTTGATGGATTAAAGTCT  300
CTTCGATTACTTTCTCTACATGGAAATGACATTTCTGTTGTGCCTGAAGGTGCTTTCAATGATCTTTCTGCATTA  375
TCACATCTAGCAATTGGAGCCAACCCTCTTTACTGTGATTGTAACATGCAGTGGTTATCCGACTGGGTGAAGTCG  450
GAATATAAGGAGCCTGGAATTGCTCGTTGTGCTGGTCCTGGAGAAATGGCAGATAAACTTTTACTCACAACTCCC  525
TCCAAAAAATTTACCTGTCAAGGTCCTGTGGATGTCAATATTCTAGCTAAGTGTAACCCC                 585
```

Hybridisation Probe for EGF repeats one to five

2752-3327

```
TGCCTATCAAATCCGTGTAAAAATGATGGCACATGTAATAGTGATCCAGTTGACTTTTACCGATGCACCTGTCCA   75
TATGGTTTCAAGGGGCAGGACTGTGATGTCCCAATTCATGCCTGCATCAGTAACCCATGTAAACATGGAGGAACT  150
TGCCACTTAAAGGAAGGAGAAGAAGATGGATTCTGGTGTATTTGTGCTGATGGATTTGAAGGAGAAAATTGTGAA  225
GTCAACGTTGATGATTGTGAAGATAATGACTGTGAAAATAATTCTACATGTGTCGATGGCATTAATAACTACACA  300
TGCCTTTGCCCACCTGAGTATACAGGTGAGTTGTGTGAGGAGAAGCTGGACTTCTGTGCCCAGGACCTGAACCCC  375
TGCCAGCACGATTCAAAGTGCATCCTAACTCCAAAGGGATTCAAATGTGACTGCACACCAGGGTACGTAGGTGAA  450
CACTGCGACATCGATTTTGACGACTGCCAAGACAACAAGTGTAAAAACGGAGCCCACTGCACAGATGCAGTGAAC  525
GGCTATACGTGCATATGCCCCGAAGGTTACAGTGGCTTGTTCTGTGAGTTT                          576
```

TABLE 5(B)

Hybridisation Probe for the sixth EGF repeat and preceding spacer region

```
TCTCCACCCATGGTCCTCCCTCGTACCAGCCCCTGTGATAATTTTGATTGTCAGAATGGAGCTCAGTGTATCGTC    75
AGAATAAATGAGCCAATATGTCAGTGTTTGCCTGGCTATCAGGGAGAAAAGTGTGAAAA                    134
```

3328 – 3461

Hybridisation Probe for the 99aa spacer/G-loop region

```
ATTGGTTAGTGTGAATTTTATAAACAAAGAGTCTTATCTTCAGATTCCTTCAGCCAAGGTTCGGCCTCAGACGAA    75
CATAACACTTCAGATTGCCACAGATGAAGACAGCGGAATCCTCCTGTATAAGGGTGACAAAGACCATATCGCGGT   150
AGAACTCTATCGGGGGCGTGTTCGTGCCAGCTATGACACCGGCTCTCATCCAGCTTCTGCCATTTACAGTGTGGA   225
GACAATCAATGATGGAAACTTCCACATTGTGGAACTACTTGCCTTGGATCAGAGTCTCTCTTTGTCCGTGGATGG   300
TGGGAACCCCAAAATCATCACTAACTTGTCAAAGCAGTCCACTCTGAATTTTGACTCTCCACTCTATGTAGGAGG   375
CATGCCAGGGAAGAGTAACGTGGCATCTCTGCGCCAGGCCCCTGGGCAGAACGGAACCAGCTTCCACGGCTGCAT   450
CCGGAACCTTTACATCAACAGTGAGCTGCAGGACTTCCAGAAGGTGCCGATGCAAACAGGCATTTTGCCTGGCTGT  526
```

3462 – 3987

Hybridisation Probe for EGF repeats seven to nine

```
GAGCCATGCCACAAGAAGGTGTGTGCCCATGGCACATGCCAGCCCAGCAGCCAGGCAGGCTTCACCTGCGAGTGC    75
CAGGAAGGATGGATGGGGCCCCTCTGTGACCAACGGACCAATGACCCTTGCCTTGGAAATAAATGCGTACATGGC   150
ACCTGCTTGCCCATCAATGCGTTCTCCTACAGCTGTAAGTGCTTGGAGGGCCATGGAGGTGTCCTCTGTGATGAA   225
GAGGAGGATCTGTTTAACCCATGCCAGGCGATCAAGTGCAAGCATGGGAAGTGCAGGCTTTCAGGTCTGGGGCAG   300
CCCTACTGTGAATGCAGCAGTGGATACACGGGGACAGCTGTGATCGAGAAATC                          353
```

3988 – 4341

Hybridisation Probe for the cysteine knot region

```
TCTTGTCGAGGGGAAAGGATAAGAGATTATTACCAAAAGCAGCAGGGCTATGCTGCTTGCCAAACAACCAAGAAG    75
GTGTCCCGATTAGAGTGCAGAGGTGGGTGTGCAGGAGGGCAGTGCTGTGGACCGCTGAGGAGCAAGCGGCGGAAA   150
TACTCTTTCGAATGCACTGACGGCTCCTCCTTTGTGGACGAGGTTGAGAAAGTGGTGAAGTGCGGCTGTACGAGG   225
TGTGTGTCC                                                                      234
```

4342 – 4575

TABLE 6

PCR Primers for regions of Human Slit-1

PCR Primers for the first Leucine rich repeat region

Forward: 5' TGCCCGGCGCAGTGCTCTTGCTCGGGCAGC 3' 82 - 111
Reverse: 5' GTGCAAAACACTACAAGAAGGAGCCATAAA 3' 799 - 828 (RC)

PCR Primers for the second Leucine rich repeat region

Forward: 5' TGCCCTGCCGCCTGTACCTGTAGCAACAAT 3' 829 - 858
Reverse: 5' AGCCAGATCCGCAAAGCAGTCTCCACTTAA 3' 1474 - 1503 RC

PCR Primers for the third Leucine rich repeat region

Forward: 5' TGCCCTGAAAAGTGTCGCTGTGAAGGAACC 3' 1504 - 1533
Reverse: 5' GCGAGAAAGTGGGGAGCAACTATTGTCATC 3' 2137 - 2166

PCR Primers for the fourth Leucine rich repeat region

Forward: 5' TGTCCTACTGAATGTACTTGCTTGGATACA 3' 2167 - 2196
Reverse: 5' GGGGTTACACTTAGCTAGAATATTGACATC 3' 2722 - 2751

PCR Primers for EGF repeats one to five

Forward: 5' TGCCTATCAAATCCGTGTAAAAATGATGGC 3' 2752 - 2781
Reverse: 5' AAACTCACAGAACAAGCCACTGTAACCTTC 3' 3298 - 3327

PCR Primers for the sixth EGF repeat and preceding spacer region

Forward: 5' TCTCCACCCATGGTCCTCCCTCGTACCAGC 3' 3328 - 3357
Reverse: 5' TTTTCACACTTTTCTCCCTGATAGCCAGGC 3' 3432 - 3461

PCR Primers for the 99aa spacer/G-loop region

Forward: 5' ATTGGTTAGTGTGAATTTTATAAACAAAGA 3' 3462 - 3491
Reverse: 5' ACAGCCAGGCAAAATGCCTGTTTGCATCGG 3' 3958 - 3987

PCR Primers for EGF repeats seven to nine

Forward: 5' GAGCCATGCCACAAGAAGGTGTGTGCCCAT 3' 3988 - 4017
Reverse: 5' GATTTCTCGATCACAGCTGTCCCCGTGTAT 3' 4312 - 4341

PCR Primers for the cysteine knot region

Forward: 5' TCTTGTCGAGGGGAAAGGATAAGAGATTAT 3' 4342 - 4371
Reverse: 5' GGACACACACCTCGTACAGCCGCACTTCAC 3' 4546 - 4575

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled *Modulating Robo: Ligand Interactions*, described in the specification filed on November 13, 1998, and having U.S. Serial No. 09/191,647.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Patent Office all information known to me to be material to patentability as defined in 37 C.F.R. 1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Patent Office all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

USSN 60/065.544 filed on November 14, 1997, abandoned, and 60/081,057 filed on April 7, 1998, pending.

Direct all telephone calls to Richard Osman (650) 343-4341 and address all correspondence to: Science & Technology Law Group, 75 Denise Drive, Hillsborough, CA 94010

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18, United States Code, §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: Corey S. Goodman

Inventor's signature: [signature]

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Residence: Berkeley, CA

Citizenship: U.S.A.

Post Office Address: Life Sciences Addition #519, UC Berkeley, Berkeley, CA 94720

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Inventor's signature:	Tom Kidd
Date:	9/17/99
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Citizenship:	U.S.A.
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Full name of third inventor:	Katja Brose
Inventor's signature:	Katja Brose
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Post Office Address:	Department of Anatomy, UCSF, 513 Parnassus Ave, Rm. S-1479, San Francisco, CA 94143-0452

Full name of fourth inventor:	Marc Tessier-Lavigne
Inventor's signature:	Marc Tessier-Lavigne
Date:	9/17/99
Residence:	San Francisco, CA
Citizenship:	Canada
Post Office Address:	Department of Anatomy, UCSF, 513 Parnassus Ave, Rm. S-1479, San Francisco, CA 94143-0452

Applicant: Goodman et al.
Serial No.: 09/191,647
Filed: November 13, 1998
Group: 1636

UCB98-031-3

POWER OF ATTORNEY BY ASSIGNEE

To the Assistant Commissioner for Patents:

The undersigned assignee of the entire interest in application for letters patent entitled: *Modulating Robo: Ligand Interactions* and having the named inventor(s):Corey S. Goodman, Thomas Kidd, Katja Brose and Marc Tessier-Lavigne, described in the application filed on November 13, 1998 having US Serial No.: 09/191,647, hereby appoints Richard Aron Osman, Ph.D. (Reg No 36,627) to prosecute this application and to transact all business in the United States Patent and Trademark Office in connection therewith.

Please direct all correspondence and telephone calls to: Richard Aron Osman, Ph.D. at 75 Denise Drive, Hillsborough, CA 94010; tel. (650) 343-4341.

In accordance with 37 CFR §3.73 the assignee submits herewith for recordation an assignment from the inventors to the undersigned assignee and hereby certifies that the evidentiary documents with respect to their ownership have been reviewed and that, to the best of assignee's knowledge and belief, title is in the assignee seaking to take this action.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application nor any patent issuing thereon.

By: The Regents of the University of California
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Signature: __________ Date: Feb 16 1999

SEQUENCE LISTING

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Kid, Thomas
Brose, Katja
Tessier-Lavigne, Marc

<120> Modulating Robo: Ligand Interactions

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  1               5                  10                  15

ctg gcg atc ctg aac aag gtg gca ccg cag gcg tgc ccg gcg cag tgc      96
Leu Ala Ile Leu Asn Lys Val Ala Pro Gln Ala Cys Pro Ala Gln Cys
             20                  25                  30

tct tgc tcg ggc agc aca gtg gac tgt cac ggg ctg gcg ctg cgc agc     144
Ser Cys Ser Gly Ser Thr Val Asp Cys His Gly Leu Ala Leu Arg Ser
         35                  40                  45

gtg ccc agg aat atc ccc cgc aac acc gag aga ctg gat tta aat gga     192
Val Pro Arg Asn Ile Pro Arg Asn Thr Glu Arg Leu Asp Leu Asn Gly
     50                  55                  60

aat aac atc aca aga att acg aag aca gat ttt gct ggt ctt aga cat     240
Asn Asn Ile Thr Arg Ile Thr Lys Thr Asp Phe Ala Gly Leu Arg His
 65                  70                  75                  80

cta aga gtt ctt cag ctt atg gag aat aag att agc acc att gaa aga     288
Leu Arg Val Leu Gln Leu Met Glu Asn Lys Ile Ser Thr Ile Glu Arg
                 85                  90                  95

gga gca ttc cag gat ctt aaa gaa cta gag aga ctg cgt tta aac aga     336
Gly Ala Phe Gln Asp Leu Lys Glu Leu Glu Arg Leu Arg Leu Asn Arg

            100                 105                 110

aat cac ctt cag ctg ttt cct gag ttg ctg ttt ctt ggg act gcg aag     384
Asn His Leu Gln Leu Phe Pro Glu Leu Leu Phe Leu Gly Thr Ala Lys
        115                 120                 125

cta tac agg ctt gat ctc agt gaa aac caa att cag gca atc cca agg     432
Leu Tyr Arg Leu Asp Leu Ser Glu Asn Gln Ile Gln Ala Ile Pro Arg
```

```
    130                 135                 140

aaa gct ttc cgt ggg gca gtt gac ata aaa aat ttg caa ctg gat tac      480
Lys Ala Phe Arg Gly Ala Val Asp Ile Lys Asn Leu Gln Leu Asp Tyr
145                 150                 155                 160

aac cag atc agc tgt att gaa gat ggg gca ttc agg gct ctc cgg gac      528
Asn Gln Ile Ser Cys Ile Glu Asp Gly Ala Phe Arg Ala Leu Arg Asp
                165                 170                 175

ctg gaa gtg ctc act ctc aac aat aac aac att act aga ctt tct gtg      576
Leu Glu Val Leu Thr Leu Asn Asn Asn Asn Ile Thr Arg Leu Ser Val
            180                 185                 190

gca agt ttc aac cat atg cct aaa ctt agg act ttt cga ctg cat tca      624
Ala Ser Phe Asn His Met Pro Lys Leu Arg Thr Phe Arg Leu His Ser
        195                 200                 205

aac aac ctg tat tgt gac tgc cac ctg gcc tgg ctc tcc gac tgg ctt      672
Asn Asn Leu Tyr Cys Asp Cys His Leu Ala Trp Leu Ser Asp Trp Leu
    210                 215                 220

cgc aaa agg cct cgg gtt ggt ctg tac act cag tgt atg ggc ccc tcc      720
Arg Lys Arg Pro Arg Val Gly Leu Tyr Thr Gln Cys Met Gly Pro Ser
225                 230                 235                 240

cac ctg aga ggc cat aat gta gcc gag gtt caa aaa cga gaa ttt gtc      768
His Leu Arg Gly His Asn Val Ala Glu Val Gln Lys Arg Glu Phe Val
                245                 250                 255

tgc agt gat gag gaa gaa ggt cac cag tca ttt atg gct cct tct tgt      816
Cys Ser Asp Glu Glu Glu Gly His Gln Ser Phe Met Ala Pro Ser Cys
            260                 265                 270

agt gtt ttg cac tgc cct gcc gcc tgt acc tgt agc aac aat atc gta      864
Ser Val Leu His Cys Pro Ala Ala Cys Thr Cys Ser Asn Asn Ile Val
        275                 280                 285

gac tgt cgt ggg aaa ggt ctc act gag atc ccc aca aat ctt cca gag      912
Asp Cys Arg Gly Lys Gly Leu Thr Glu Ile Pro Thr Asn Leu Pro Glu
    290                 295                 300

acc atc aca gaa ata cgt ttg gaa cag aac aca atc aaa gtc atc cct      960
Thr Ile Thr Glu Ile Arg Leu Glu Gln Asn Thr Ile Lys Val Ile Pro
305                 310                 315                 320

cct gga gct ttc tca cca tat aaa aag ctt aga cga att gac ctg agc     1008
Pro Gly Ala Phe Ser Pro Tyr Lys Lys Leu Arg Arg Ile Asp Leu Ser
                325                 330                 335

aat aat cag atc tct gaa ctt gca cca gat gct ttc caa gga cta cgc     1056
Asn Asn Gln Ile Ser Glu Leu Ala Pro Asp Ala Phe Gln Gly Leu Arg

            340                 345                 350

tct ctg aat tca ctt gtc ctc tat gga aat aaa atc aca gaa ctc ccc     1104
Ser Leu Asn Ser Leu Val Leu Tyr Gly Asn Lys Ile Thr Glu Leu Pro
        355                 360                 365

aaa agt tta ttt gaa gga ctg ttt tcc tta cag ctc cta tta ttg aat     1152
Lys Ser Leu Phe Glu Gly Leu Phe Ser Leu Gln Leu Leu Leu Leu Asn
    370                 375                 380

gcc aac aag ata aac tgc ctt cgg gta gat gct ttt cag gat ctc cac     1200
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Glu Asn Gln Asp Asp Cys Arg Asp His Arg Cys Gln Asn Gly Ala Gln
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Cys Met Asp Glu Val Asn Ser Tyr Ser Cys Leu Cys Ala Glu Gly Tyr
        35   40   45

Ser Gly Gln Leu Cys Glu Ile Pro Pro His Leu Pro Ala Pro Lys Ser
    50   55   60

Pro Cys Glu Gly Thr Glu Cys Gln Asn Gly Ala Asn Cys Val Asp Gln
65   70   75   80

Gly Asn Arg Pro Val Cys Gln Cys Leu Pro Gly Phe Gly Gly Pro Glu
                85   90   95

Cys Glu Lys Leu Leu Ser Val Asn Phe Val Asp Arg Asp Thr Tyr Leu
            100   105   110

Gln Phe Thr Asp Leu Gln Asn Trp Xaa Arg Xaa Asn Ile Thr Leu Gln
        115   120   125

Val Phe Thr Ala Glu Asp Asn Gly Ile Leu Leu Tyr Asn Gly Gly Asn
    130   135   140

Asp His Ile Ala Val Xaa Leu Tyr Xaa Gly His Val Arg Phe Ser Tyr

145 150 155 160

<210> 6
<211> 103
<212> PRT
<213> human

<400> 6
Gln Cys His Ile Ser Asp Gln Gly Glu Pro Tyr Cys Leu Cys Gln Pro
1 5 10 15

Gly Phe Ser Gly Glu His Cys Gln Gln Glu Asn Pro Cys Leu Gly Gln
20 25 30

Val Val Arg Glu Val Ile Arg Arg Gln Lys Gly Tyr Ala Ser Cys Ala
35 40 45

Thr Ala Ser Lys Val Pro Ile Met Glu Cys Arg Gly Gly Cys Gly Pro
50 55 60

Gln Cys Cys Gln Pro Thr Arg Ser Lys Arg Arg Lys Tyr Val Phe Gln
65 70 75 80

Cys Thr Asp Gly Ser Ser Phe Val Glu Glu Val Glu Arg His Leu Glu
85 90 95

Cys Gly Cys Leu Ala Cys Ser
100

<210> 7
<211> 1480
<212> PRT
<213> Drosophila melanogaster

<400> 7
Met Ala Ala Pro Ser Arg Thr Thr Leu Met Pro Pro Pro Phe Arg Leu
1 5 10 15

Gln Leu Arg Leu Leu Ile Leu Pro Ile Leu Leu Leu Leu Arg His Asp
20 25 30

Ala Val His Ala Glu Pro Tyr Ser Gly Gly Phe Gly Ser Ser Ala Val
35 40 45

Ser Ser Gly Gly Leu Gly Ser Val Gly Ile His Ile Pro Gly Gly Gly
50 55 60

Val Gly Val Ile Thr Glu Ala Arg Cys Pro Arg Val Cys Ser Cys Thr
65 70 75 80

Gly Leu Asn Val Asp Cys Ser His Arg Gly Leu Thr Ser Val Pro Arg
85 90 95

Lys Ile Ser Ala Asp Val Glu Arg Leu Glu Leu Gln Gly Asn Asn Leu
100 105 110

Thr Val Ile Tyr Glu Thr Asp Phe Gln Arg Leu Thr Lys Leu Arg Met
115 120 125

Leu Gln Leu Thr Asp Asn Gln Ile His Thr Ile Glu Arg Asn Ser Phe
130 135 140

Gln Asp Leu Val Ser Leu Glu Arg Leu Asp Ile Ser Asn Asn Val Ile

145 150 155 160

Thr Thr Val Gly Arg Arg Val Phe Lys Gly Ala Gln Ser Leu Arg Ser
165 170 175

Leu Gln Leu Asp Asn Asn Gln Ile Thr Cys Leu Asp Glu His Ala Phe
180 185 190

Lys Gly Leu Val Glu Leu Glu Ile Leu Thr Leu Asn Asn Asn Asn Leu
195 200 205

Thr Ser Leu Pro His Asn Ile Phe Gly Gly Leu Gly Arg Leu Arg Ala
210 215 220

Leu Arg Leu Ser Asp Asn Pro Phe Ala Cys Asp Cys His Leu Ser Trp
225 230 235 240

Leu Ser Arg Phe Leu Arg Ser Ala Thr Arg Leu Ala Pro Tyr Thr Arg
245 250 255

Cys Gln Ser Pro Ser Gln Leu Lys Gly Gln Asn Val Ala Asp Leu His
260 265 270

Asp Gln Glu Phe Lys Cys Ser Gly Leu Thr Glu His Ala Pro Met Glu
275 280 285

Cys Gly Ala Glu Asn Ser Cys Pro His Pro Cys Arg Cys Ala Asp Gly
290 295 300

Ile Val Asp Cys Arg Glu Lys Ser Leu Thr Ser Val Pro Val Thr Leu
305 310 315 320

Pro Asp Asp Thr Thr Asp Val Arg Leu Glu Gln Asn Phe Ile Thr Glu
325 330 335

Leu Pro Pro Lys Ser Phe Ser Ser Phe Arg Arg Leu Arg Arg Ile Asp
340 345 350

Leu Ser Asn Asn Asn Ile Ser Arg Ile Ala His Asp Ala Leu Ser Gly
355 360 365

Leu Lys Gln Leu Thr Thr Leu Val Leu Tyr Gly Asn Lys Ile Lys Asp
370 375 380

Leu Pro Ser Gly Val Phe Lys Gly Leu Gly Ser Leu Arg Leu Leu Leu
385 390 395 400

Leu Asn Ala Asn Glu Ile Ser Cys Ile Arg Lys Asp Ala Phe Arg Asp
405 410 415

Leu His Ser Leu Ser Leu Leu Ser Leu Tyr Asp Asn Asn Ile Gln Ser
420 425 430

Leu Ala Asn Gly Thr Phe Asp Ala Met Lys Ser Met Lys Thr Val His
435 440 445

Leu Ala Lys Asn Pro Phe Ile Cys Asp Cys Asn Leu Arg Trp Leu Ala
450 455 460

Asp Tyr Leu His Lys Asn Pro Ile Glu Thr Ser Gly Ala Arg Cys Glu
465 470 475 480

Ser Pro Lys Arg Met His Arg Arg Arg Ile Glu Ser Leu Arg Glu Glu
485 490 495

Lys Phe Lys Cys Ser Trp Gly Glu Leu Arg Met Lys Leu Ser Gly Glu
500 505 510

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Cys Arg Met Asp Ser Asp Cys Pro Ala Met Cys His Cys Glu Gly Thr
        515                 520                 525

Thr Val Asp Cys Thr Gly Arg Arg Leu Lys Glu Ile Pro Arg Asp Ile
    530                 535                 540

Pro Leu His Thr Thr Glu Leu Leu Leu Asn Asp Asn Glu Leu Gly Arg
545                 550                 555                 560

Ile Ser Ser Asp Gly Leu Phe Gly Arg Leu Pro His Leu Val Lys Leu
                565                 570                 575

Glu Leu Lys Arg Asn Gln Leu Thr Gly Ile Glu Pro Asn Ala Phe Glu
            580                 585                 590

Gly Ala Ser His Ile Gln Glu Leu Gln Leu Gly Glu Asn Lys Ile Lys
        595                 600                 605

Glu Ile Ser Asn Lys Met Phe Leu Gly Leu His Gln Leu Lys Thr Leu
    610                 615                 620

Asn Leu Tyr Asp Asn Gln Ile Ser Cys Val Met Pro Gly Ser Phe Glu
625                 630                 635                 640

His Leu Asn Ser Leu Thr Ser Leu Asn Leu Ala Ser Asn Pro Phe Asn
                645                 650                 655

Cys Asn Cys His Leu Ala Trp Phe Ala Glu Cys Val Arg Lys Lys Ser
            660                 665                 670

Leu Asn Gly Gly Ala Ala Arg Cys Gly Ala Pro Ser Lys Val Arg Asp
        675                 680                 685

Val Gln Ile Lys Asp Leu Pro His Ser Glu Phe Lys Cys Ser Ser Glu
    690                 695                 700

Asn Ser Glu Gly Cys Leu Gly Asp Gly Tyr Cys Pro Pro Ser Cys Thr
705                 710                 715                 720

Cys Thr Gly Thr Val Val Ala Cys Ser Arg Asn Gln Leu Lys Glu Ile
                725                 730                 735

Pro Arg Gly Ile Pro Ala Glu Thr Ser Glu Leu Tyr Leu Glu Ser Asn
            740                 745                 750

Glu Ile Glu Gln Ile His Tyr Glu Arg Ile Arg His Leu Arg Ser Leu
        755                 760                 765

Thr Arg Leu Asp Leu Ser Asn Asn Gln Ile Thr Ile Leu Ser Asn Tyr
    770                 775                 780

Thr Phe Ala Asn Leu Thr Lys Leu Ser Thr Leu Ile Ile Ser Tyr Asn
785                 790                 795                 800

Lys Leu Gln Cys Leu Gln Arg His Ala Leu Ser Gly Leu Asn Asn Leu
                805                 810                 815

Arg Val Val Ser Leu His Gly Asn Arg Ile Ser Met Leu Pro Glu Gly
            820                 825                 830

Ser Phe Glu Asp Leu Lys Ser Leu Thr His Ile Ala Leu Gly Ser Asn
        835                 840                 845

Pro Leu Tyr Cys Asp Cys Gly Leu Lys Trp Phe Ser Asp Trp Ile Lys
    850                 855                 860

Leu Asp Tyr Val Glu Pro Gly Ile Ala Arg Cys Ala Glu Pro Glu Gln
865                 870                 875                 880
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Met Lys Asp Lys Leu Ile Leu Ser Thr Pro Ser Ser Ser Phe Val Cys
885 890 895

Arg Gly Arg Val Arg Asn Asp Ile Leu Ala Lys Cys Asn Ala Cys Phe
900 905 910

Glu Gln Pro Cys Gln Asn Gln Ala Gln Cys Val Ala Leu Pro Gln Arg
915 920 925

Glu Tyr Gln Cys Leu Cys Gln Pro Gly Tyr His Gly Lys His Cys Glu
930 935 940

Phe Met Ile Asp Ala Cys Tyr Gly Asn Pro Cys Arg Asn Asn Ala Thr
945 950 955 960

Cys Thr Val Leu Glu Glu Gly Arg Phe Ser Cys Gln Cys Ala Pro Gly
965 970 975

Tyr Thr Gly Ala Arg Cys Glu Thr Asn Ile Asp Asp Cys Leu Gly Glu
980 985 990

Ile Lys Cys Gln Asn Asn Ala Thr Cys Ile Asp Gly Val Glu Ser Tyr
995 1000 1005

Lys Cys Glu Cys Gln Pro Gly Phe Ser Gly Glu Phe Cys Asp Thr Lys
1010 1015 1020

Ile Gln Phe Cys Ser Pro Glu Phe Asn Pro Cys Ala Asn Gly Ala Lys
1025 1030 1035 1040

Cys Met Asp His Phe Thr His Tyr Ser Cys Asp Cys Gln Ala Gly Phe
1045 1050 1055

His Gly Thr Asn Cys Thr Asp Asn Ile Asp Asp Cys Gln Asn His Met
1060 1065 1070

Cys Gln Asn Gly Gly Thr Cys Val Asp Gly Ile Asn Asp Tyr Gln Cys
1075 1080 1085

Arg Cys Pro Asp Asp Tyr Thr Gly Lys Tyr Cys Glu Gly His Asn Met
1090 1095 1100

Ile Ser Met Met Tyr Pro Gln Thr Ser Pro Cys Gln Asn His Glu Cys
1105 1110 1115 1120

Lys His Gly Val Cys Phe Gln Pro Asn Ala Gln Gly Ser Asp Tyr Leu
1125 1130 1135

Cys Arg Cys His Pro Gly Tyr Thr Gly Lys Trp Cys Glu Tyr Leu Thr
1140 1145 1150

Ser Ile Ser Phe Val His Asn Asn Ser Phe Val Glu Leu Glu Pro Leu
1155 1160 1165

Arg Thr Arg Pro Glu Ala Asn Val Thr Ile Val Phe Ser Ser Ala Glu
1170 1175 1180

Gln Asn Gly Ile Leu Met Tyr Asp Gly Gln Asp Ala His Leu Ala Val
1185 1190 1195 1200

Glu Leu Phe Asn Gly Arg Ile Arg Val Ser Tyr Asp Val Gly Asn His
1205 1210 1215

Pro Val Ser Thr Met Tyr Ser Phe Glu Met Val Ala Asp Gly Lys Tyr
1220 1225 1230

His Ala Val Glu Leu Leu Ala Ile Lys Lys Asn Phe Thr Leu Arg Val
1235 1240 1245

Asp Arg Gly Leu Ala Arg Ser Ile Ile Asn Glu Gly Ser Asn Asp Tyr
1250 1255 1260

Leu Lys Leu Thr Thr Pro Met Phe Leu Gly Gly Leu Pro Val Asp Pro
1265 1270 1275 1280

Ala Gln Gln Ala Tyr Lys Asn Trp Gln Ile Arg Asn Leu Thr Ser Phe
1285 1290 1295

Lys Gly Cys Met Lys Glu Val Trp Ile Asn His Lys Leu Val Asp Phe
1300 1305 1310

Gly Asn Ala Gln Arg Gln Gln Lys Ile Thr Pro Gly Cys Ala Leu Leu
1315 1320 1325

Glu Gly Glu Gln Gln Glu Glu Glu Asp Asp Glu Gln Asp Phe Met Asp
1330 1335 1340

Glu Thr Pro His Ile Lys Glu Glu Pro Val Asp Pro Cys Leu Glu Asn
1345 1350 1355 1360

Lys Cys Arg Arg Gly Ser Arg Cys Val Pro Asn Ser Asn Ala Arg Asp
1365 1370 1375

Gly Tyr Gln Cys Lys Cys Lys His Gly Gln Arg Gly Arg Tyr Cys Asp
1380 1385 1390

Gln Gly Glu Gly Ser Thr Glu Pro Pro Thr Val Thr Ala Ala Ser Thr
1395 1400 1405

Cys Arg Lys Glu Gln Val Arg Glu Tyr Tyr Thr Glu Asn Asp Cys Arg
1410 1415 1420

Ser Arg Gln Pro Leu Lys Tyr Ala Lys Cys Val Gly Gly Cys Gly Asn
1425 1430 1435 1440

Gln Cys Cys Ala Ala Lys Ile Val Arg Arg Arg Lys Val Arg Met Val
1445 1450 1455

Cys Ser Asn Asn Arg Lys Tyr Ile Lys Asn Leu Asp Ile Val Arg Lys
1460 1465 1470

Cys Gly Cys Thr Lys Lys Cys Tyr
1475 1480

<210> 8
<211> 155
<212> PRT
<213> Caenorhabditis elegans

<400> 8
Arg Asn Pro Xaa Ile Cys Asp Cys Asn Leu Gln Trp Leu Ala Gln Ile
1 5 10 15

Asn Leu Gln Lys Asn Ile Glu Thr Ser Gly Ala Arg Cys Glu Gln Pro
20 25 30

Lys Arg Leu Arg Lys Lys Lys Phe Ala Thr Leu Pro Pro Asn Lys Phe
35 40 45

Lys Cys Lys Gly Ser Glu Ser Phe Val Ser Met Tyr Ala Asp Ser Cys
50 55 60

Phe Ile Asp Ser Ile Cys Pro Thr Gln Cys Asp Cys Tyr Gly Thr Thr

65 70 75 80

Val Asp Cys Asn Lys Arg Gly Leu Asn Thr Ile Pro Thr Ser Ile Pro
85 90 95

Arg Phe Ala Thr Gln Leu Leu Leu Ser Gly Asn Asn Ile Ser Thr Val
100 105 110

Asp Leu Asn Ser Asn Ile His Val Leu Glu Asn Leu Glu Xaa Leu Asp
115 120 125

Leu Ser Asn Asn His Ile Thr Phe Ile Asn Asp Lys Ser Phe Glu Lys
130 135 140

Leu Ser Lys Leu Arg Glu Leu Xaa Leu Asn Asp
145 150 155

<210> 9
<211> 735
<212> PRT
<213> Caenorhabditis elegans

<400> 9
Ser Asn Lys Asn Leu Thr Ser Phe Pro Ser Arg Ile Pro Phe Asp Thr
1 5 10 15

Thr Glu Leu Tyr Leu Asp Ala Asn Tyr Ile Asn Glu Ile Pro Ala His
20 25 30

Asp Leu Asn Arg Leu Tyr Ser Leu Thr Lys Leu Asp Leu Ser His Asn
35 40 45

Arg Leu Ile Ser Leu Glu Asn Asn Thr Phe Ser Asn Leu Thr Arg Leu
50 55 60

Ser Thr Leu Ile Ile Ser Tyr Asn Lys Leu Arg Cys Leu Gln Pro Leu
65 70 75 80

Ala Phe Asn Gly Leu Asn Ala Leu Arg Ile Leu Ser Leu His Gly Asn
85 90 95

Asp Ile Ser Phe Leu Pro Gln Ser Ala Phe Ser Asn Leu Thr Ser Ile
100 105 110

Thr His Ile Ala Val Gly Ser Asn Ser Leu Tyr Cys Asp Cys Asn Met
115 120 125

Ala Trp Phe Ser Lys Trp Ile Lys Ser Lys Phe Ile Glu Ala Gly Ile
130 135 140

Ala Arg Cys Glu Tyr Pro Asn Thr Val Ser Asn Gln Leu Leu Leu Thr
145 150 155 160

Ala Gln Pro Tyr Gln Phe Thr Cys Asp Ser Lys Val Pro Thr Lys Leu
165 170 175

Ala Thr Lys Cys Asp Leu Cys Leu Asn Ser Pro Cys Lys Asn Asn Ala
180 185 190

Ile Cys Glu Thr Thr Ser Ser Arg Lys Tyr Thr Cys Asn Cys Thr Pro
195 200 205

Gly Phe Tyr Gly Val His Cys Glu Asn Gln Ile Asp Ala Cys Tyr Gly
210 215 220

Ser Pro Cys Leu Asn Asn Ala Thr Cys Lys Val Ala Gln Ala Gly Arg
225 230 235 240

Phe Asn Cys Tyr Cys Asn Lys Gly Phe Glu Gly Asp Tyr Cys Glu Lys
                245   250   255

Asn Ile Asp Asp Cys Val Asn Ser Lys Cys Glu Asn Gly Gly Lys Cys
            260   265   270

Val Asp Leu Val Arg Phe Cys Ser Glu Glu Leu Lys Asn Phe Gln Ser
        275   280   285

Phe Gln Ile Asn Ser Tyr Arg Cys Asp Cys Pro Met Glu Tyr Glu Gly
    290   295   300

Lys His Cys Glu Asp Lys Leu Glu Tyr Cys Thr Lys Lys Leu Asn Pro
305   310   315   320

Cys Glu Asn Asn Gly Lys Cys Ile Pro Ile Asn Gly Ser Tyr Ser Cys
                325   330   335

Met Cys Ser Pro Gly Phe Thr Gly Asn Asn Cys Glu Thr Asn Ile Asp
            340   345   350

Asp Cys Lys Asn Val Glu Cys Gln Asn Gly Gly Ser Cys Val Asp Gly
        355   360   365

Ile Leu Ser Tyr Asp Cys Leu Cys Arg Pro Gly Tyr Ala Gly Gln Tyr
    370   375   380

Cys Glu Ile Pro Pro Met Met Asp Met Glu Tyr Gln Lys Thr Asp Ala
385   390   395   400

Cys Gln Gln Ser Ala Cys Gly Gln Gly Glu Cys Val Ala Ser Gln Asn
                405   410   415

Ser Ser Asp Phe Thr Cys Lys Cys His Glu Gly Phe Ser Gly Pro Ser
            420   425   430

Cys Asp Arg Gln Met Ser Val Gly Phe Lys Asn Pro Gly Ala Tyr Leu
        435   440   445

Ala Leu Asp Pro Leu Ala Ser Asp Gly Thr Ile Thr Met Thr Leu Arg
    450   455   460

Thr Thr Ser Lys Ile Gly Ile Leu Leu Tyr Tyr Gly Asp Asp His Phe
465   470   475   480

Val Ser Ala Glu Leu Tyr Asp Gly Arg Val Lys Leu Val Tyr Tyr Ile
                485   490   495

Gly Asn Phe Pro Ala Ser His Met Tyr Ser Ser Val Lys Val Asn Asp
            500   505   510

Gly Leu Pro His Arg Ile Ser Ile Arg Thr Ser Glu Arg Lys Cys Phe
        515   520   525

Leu Gln Ile Asp Lys Asn Pro Val Gln Ile Val Glu Asn Ser Gly Lys
    530   535   540

Ser Asp Gln Leu Ile Thr Lys Gly Lys Glu Met Leu Tyr Ile Gly Gly
545   550   555   560

Leu Pro Ile Glu Lys Ser Gln Asp Ala Lys Arg Arg Phe His Val Lys
                565   570   575

Asn Ser Glu Ser Leu Lys Gly Cys Ile Ser Ser Ile Thr Ile Asn Glu
            580   585   590

Val Pro Ile Asn Leu Gln Gln Ala Leu Glu Asn Val Asn Thr Glu Gln

595 600 605

Ser Cys Ser Ala Thr Val Asn Phe Cys Ala Gly Ile Asp Cys Gly Asn
610 615 620

Gly Lys Cys Thr Asn Asn Ala Leu Ser Pro Lys Gly Tyr Met Cys Gln
625 630 635 640

Cys Asp Ser His Phe Ser Gly Glu His Cys Asp Glu Lys Arg Ile Lys
645 650 655

Cys Asp Lys Gln Lys Phe Arg Arg His His Ile Glu Asn Glu Cys Arg
660 665 670

Ser Val Asp Arg Ile Lys Ile Ala Glu Cys Asn Gly Tyr Cys Gly Gly
675 680 685

Glu Gln Asn Cys Cys Thr Ala Val Lys Lys Lys Gln Arg Lys Val Lys
690 695 700

Met Ile Cys Lys Asn Gly Thr Thr Lys Ile Ser Thr Val His Ile Ile
705 710 715 720

Arg Gln Cys Gln Cys Glu Pro Thr Lys Ser Val Leu Ser Glu Lys
725 730 735

<210> 10
<211> 154

<212> PRT
<213> mouse

<400> 10
Asp Pro Leu Pro Val His His Arg Cys Glu Cys Met Leu Gly Tyr Thr
1 5 10 15

Gly Asp Asn Cys Ser Glu Asn Gln Asp Asp Cys Lys Asp His Lys Cys
20 25 30

Gln Asn Gly Ala Gln Cys Val Asp Glu Val Asn Ser Tyr Ala Cys Leu
35 40 45

Cys Val Glu Gly Tyr Ser Gly Gln Leu Cys Glu Ile Pro Pro Ala Pro
50 55 60

Arg Ser Ser Cys Glu Gly Thr Glu Cys Gln Asn Gly Ala Asn Cys Val
65 70 75 80

Asp Gln Gly Ser Arg Pro Val Cys Gln Cys Leu Pro Gly Phe Gly Gly
85 90 95

Pro Glu Cys Glu Lys Leu Leu Ser Val Asn Phe Val Asp Arg Asp Thr
100 105 110

Tyr Leu Gln Phe Thr Asp Leu Gln Asn Trp Pro Arg Ala Asn Ile Thr
115 120 125

Leu Gln Val Ser Thr Ala Glu Asp Asn Gly Ile Leu Leu Tyr Asn Gly
130 135 140

Asp Asn Asp His Ile Ala Val Glu Leu Tyr
145 150

<210> 11
<211> 110
<212> PRT
<213> mouse

<400> 11
Ala Phe Lys Cys His His Gly Gln Cys His Ile Ser Asp Arg Gly Glu
1 5 10 15

Pro Tyr Cys Leu Cys Gln Pro Gly Phe Ser Gly His His Cys Glu Gln
20 25 30

Glu Asn Pro Cys Met Gly Glu Ile Val Arg Glu Ala Ile Arg Arg Gln
35 40 45

Lys Asp Tyr Ala Ser Cys Ala Thr Ala Ser Lys Val Pro Ile Met Glu
50 55 60

Cys Arg Gly Gly Cys Gly Thr Thr Cys Cys Gln Pro Ile Arg Ser Lys
65 70 75 80

Arg Arg Lys Tyr Val Phe Gln Cys Thr Asp Gly Ser Ser Phe Val Glu
85 90 95

Glu Val Glu Arg His Leu Glu Cys Gly Cys Arg Ala Cys Ser

100 105 110

<210> 12
<211> 134
<212> PRT
<213> mouse

<400> 12
His Leu Arg Val Leu Gln Leu Met Glu Asn Arg Ile Ser Thr Ile Glu
1 5 10 15

Arg Gly Ala Phe Gln Asp Leu Lys Glu Leu Glu Arg Leu Arg Leu Asn
20 25 30

Arg Asn Asn Leu Gln Leu Phe Pro Glu Leu Leu Phe Leu Gly Thr Ala
35 40 45

Arg Leu Tyr Arg Leu Asp Leu Ser Glu Asn Gln Ile Gln Ala Ile Pro
50 55 60

Arg Lys Ala Phe Arg Gly Ala Val Asp Ile Lys Asn Leu Gln Leu Asp
65 70 75 80

Tyr Asn Gln Ile Ser Cys Ile Glu Asp Gly Ala Phe Arg Ala Leu Arg
85 90 95

Asp Leu Glu Val Leu Thr Leu Asn Asn Asn Asn Ile Thr Arg Leu Ser
100 105 110

Val Ala Ser Phe Asn His Met Pro Lys Leu Arg Thr Phe Arg Leu His
115 120 125

Ser Asn Asn Leu Tyr Cys
130

<210> 13
<211> 104
<212> PRT
<213> mouse

<400> 13
Asn Asn Asp Asp Cys Val Gly His Lys Cys Arg His Gly Ala Gln Cys
1 5 10 15

Val Asp Glu Val Asn Gly Tyr Thr Cys Ile Cys Pro Gln Gly Phe Ser

20 25 30

Gly Leu Phe Cys Glu His Pro Pro Pro Met Val Leu Leu Gln Thr Ser
35 40 45

Pro Cys Asp Gln Tyr Glu Cys Gln Asn Gly Ala Gln Cys Ile Val Val
50 55 60

Gln Gln Glu Pro Thr Cys Arg Cys Pro Pro Gly Phe Ala Gly Pro Arg
65 70 75 80

Cys Glu Lys Leu Ile Thr Val Asn Phe Val Gly Lys Asp Ser Tyr Val
85 90 95

Glu Leu Ala Ser Ala Lys Val Arg
100

<210> 14
<211> 243
<212> PRT
<213> mouse

<400> 14
Ile Leu Asp Val Ala Ser Leu Arg Gln Ala Pro Gly Glu Asn Gly Thr
1 5 10 15

Ser Phe His Gly Cys Ile Arg Asn Leu Tyr Ile Asn Ser Glu Leu Gln
20 25 30

Asp Phe Arg Lys Met Pro Met Gln Thr Gly Ile Leu Pro Gly Cys Glu
35 40 45

Pro Cys His Lys Lys Val Cys Ala His Gly Cys Cys Gln Pro Ser Ser
50 55 60

Gln Ser Gly Phe Thr Cys Glu Cys Glu Glu Gly Trp Met Gly Pro Leu
65 70 75 80

Cys Asp Gln Arg Thr Asn Asp Pro Cys Leu Gly Asn Lys Cys Val His
85 90 95

Gly Thr Cys Leu Pro Ile Asn Ala Phe Ser Tyr Ser Cys Lys Cys Leu
100 105 110

Glu Gly His Gly Gly Val Leu Cys Asp Glu Glu Glu Asp Leu Phe Asn
115 120 125

Pro Cys Gln Met Ile Lys Cys Lys His Gly Lys Cys Arg Leu Ser Gly
130 135 140

Val Gly Gln Pro Tyr Cys Glu Cys Asn Ser Gly Phe Thr Gly Asp Ser
145 150 155 160

Cys Asp Arg Glu Ile Ser Cys Arg Gly Glu Arg Ile Arg Asp Tyr Tyr
165 170 175

Gln Lys Gln Gln Gly Tyr Ala Ala Cys Gln Thr Thr Lys Lys Val Ser
180 185 190

Arg Leu Glu Cys Arg Gly Gly Cys Ala Gly Gly Gln Cys Cys Gly Pro
195 200 205

Leu Arg Ser Lys Arg Arg Lys Tyr Ser Phe Glu Cys Thr Asp Gly Ser
210 215 220

Ser Phe Val Asp Glu Val Glu Lys Val Val Lys Cys Gly Cys Ala Arg
225 230 235 240

Cys Ala Ser